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(71) Applicant: **QUESTCOR PHARMACEUTICALS, INC.** [US/US]; 26118 Research Road, Hayward, CA 94545 (US).

(72) Inventors: **FRECHETTE, Roger**; 40 Estate Lane, Reading, MA 01867 (US). **DAVIS, Susan**; 7 Molt Close, Bardsley LS16 8DJ (GB). **JAEGER, Chris**; 346 Tideway Drive, Alameda, CA 94501 (US). **CHONG, Lee**; 1310 Araujo Street, San Jose, CA 95131 (US). **KNAP, Ania**; 40 Estate Lane, Reading, MA 01867 (US). **WITHERELL, Gary**; 70 Ardilla Road, Orinda, CA 94563 (US). **MOEHLE, Charles**; 19100 Crest Avenue #102, Castro Valley, CA 94546 (US). **GLUCHOWSKI, Charles**; 154 Coolspring Court, Danville, CA 94506 (US).

(74) Agents: **POISSANT, Brian, M.** et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

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(54) **Title:** METHODS OF USE OF PEPTIDE DEFORMYLASE INHIBITORS AS NOVEL ANTIBACTERIAL AGENTS

(57) **Abstract:** The present invention relates to novel drug discovery assays which specifically target peptide deformylase containing the typically unstable native catalytic iron metal center. The present invention further relates to methods of identifying compounds which have greater than 10-fold, and preferably greater than 100-fold selectivity for peptide deformylase over other metalloproteases. The invention further provides for compounds having antibacterial activity identified by the assays of the invention, methods of using the compounds to treat bacterial infections and pharmaceutical compositions containing the compounds of the invention.

METHODS OF USE OF PEPTIDE DEFORMYLASE INHIBITORS AS NOVEL ANTIBACTERIAL AGENTS

1. FIELD OF INVENTION

The present invention relates to novel assays which detect compounds which selectively inhibit peptide deformylase (PDF), compounds detected in the assays, method of using the compounds and pharmaceutical compositions containing the compounds of the invention.

2. BACKGROUND OF THE INVENTION

One of the most grave problems facing mankind is the continual battle against infectious diseases. Infectious diseases are the leading cause of death worldwide and the third leading cause of death in the United States. A particularly troublesome trend that has emerged in recent years is that infectious diseases that were once susceptible to treatment with antibiotics are now becoming resistant to those antibiotics. Consider, for example, the bacterium Staphylococcus aureus, the most common cause of hospital-acquired infection. The discovery that penicillin killed bacteria led to the common use of penicillin during World War II in army hospitals. Soon after the debut of penicillin, strains of Staphylococcus aureus were isolated that were resistant to penicillin. In fact, these resistant strains were commonly found in hospitals were penicillin was heavily used. The chemically modified penicillin derivative methicillin was developed and found to be effective against penicillin resistant strains, but incredibly, soon after the introduction of methicillin, new methicillin resistant strains were isolated. Currently, many methicillin resistant Staphylococcus aureus strains have been characterized and are causing significant issues both within and outside the hospital setting. It appears that the capability of bacteria to become resistant to drugs is weakening our ability to fight bacterial infections.

New antibiotics are desperately needed as clinically significant bacterial pathogens have acquired resistance to nearly all existing antibiotics (Chopra et al., 1997, *Antimicrob. Agents Chemother* **37**:1563-1571; Cohen, 1992, *Science* **257**:1050-1055; Kunin, 1993, *Ann. Intern. Med.* **118**:557-561; Neu, 1992, *Science* **257**:1064-1073; Tenover and Hughes, 1996, *JAMA* **275**:300-304).

Bacteria acquire resistance to antimicrobial drugs through a remarkable variety of mechanisms (Russell and Chopra, 1996, Understanding antibacterial action and resistance, 2nd ed. Ellis Horwood, New York, NY; Jacobs, 1994, *Clin. Infect. Dis.* **19**:1-10; Hooper and Wolfson, 1993, *Am. Soc. Microbiol.* **1993**:97-118). The robust fitness of microorganisms is a manifestion of their short replication times and their ability to evolve in the face of the selective pressure exerted by antibiotics. In order to survive, microorganisms have developed the ability to adapt quickly and effectively to changes in the environment such as changes in the light intensity, oxygen levels, acidity and exposure to antibiotics. The emergence and spread of resistant bacteria is primarily caused by acquisition of drug resistance genes resulting in a broad spectrum of antibiotic resistance (*e.g.*, extended-spectrum cephalosporin-resistant mutant β-lactamases found in several bacterial organisms). Genetic exchange of multiple-resistance genes, by transformation, transduction and conjugation, combined with selective pressures in settings such as hospitals where there is heavy use of antibiotic therapies, has enhanced the survival and proliferation of antimicrobial agent-resistant bacterial strains occurring by, *e.g.*, spontaneous mutants. *Id.* Resistance has inevitably developed to all antimicrobial agents, although the extent to which bacteria develop resistance to antimicrobial drugs and the speed with which they do so varies types. (Gold and Moellering, Jr., 1996, *New Eng. J. Med.* **335**(19):1445-1453). Prevention of life threatening microbial infections coupled with medical practice aimed at minimizing the development of drug

resistance are certainly important (Moellering, 1990, *Scand. J. Infect. Dis.* Suppl. **70**:18-24; McCaig and Hughes, 1995, *JAMA* **273**:214-219; Guillemot, 1998, *JAMA* **279**:365-370), but to effectively battle infectious diseases, it is necessary to develop new antimicrobial drugs.

The biochemical apparatus for the synthesis of polypetides in bacteria and eukaryotes is strikingly different. It is preferable to have antimicrobial drugs that disrupt bacterial metabolism but that do not interfere with the host's metabolism. Antimicrobial drugs that interfere with the biochemical machinery of the host may cause the deleterious side effects associated with certain drugs. Thus, there is a need for antimicrobial drugs that specifically inhibit bacterial metabolism while not effecting eukaryotic biochemistry.

A significant difference between bacterial and eukaryotic protein synthesis is the use of formylated methionine as the first amino acid used during ribosome based synthesis of polypeptides. In most cases eukaryotic protein biosynthesis is initiated with methionine whereas bacterial biosynthesis commences with N-formyl methionine. Proper protein folding and function in bacteria usually occurs only after the N-formyl group is removed from the nascent polypeptide chain. This is accomplished by an enzyme called peptide deformylase.

Peptide deformylase activity in bacteria was first demonstrated over 30 years ago. Since its discovery it has proven to be a very difficult enzyme to study due to its extreme lability. The gene for PDF was recently cloned from *E. coli* and the corresponding protein was overexpressed (Meinnel and Blanquet, (1993) *J. Bacteriol.* **175**:7737-7740). Peptide deformylase is an essential enzyme for bacteria. Recent studies, directed towards understanding the biochemistry of bacteria and not drug discovery, unambiguously demonstrated that mutant strains which had their peptide deformylase gene inactivated were unable to survive (Mazel et al., (1994) *EMBO J.* **13**:914-923).

Recently, several approaches were used to characterize peptide deformylase. Peptide deformylase was the subject of efforts to determine the structural elements of peptide substrates that effect catalysis and binding. A combinatorial peptide library was used to determine kinetic and binding constant for various resin-bound peptide substrates (Hu et al.,(1999) *Biochemistry* **38**:643-650). Other studies concerning the mechanism of PDF have focused on characterizing the structural interactions between zinc and cobalt forms of the enzyme with a phosphonate transition state analogue (Hao et al.,(1999) *Biochemistry* **38**:4712-4719). Although some binding interactions were identified in this study, the use of the cobalt and zinc forms of PDF is not completely indicative of the binding interaction that govern the native iron form of PDF. These approaches have yielded important information about binding interactions between PDF and substrates but potential drugs that are selective for PDF were not identified.

Investigations of peptide deformylase have focused on the development of an assay procedure which circumvents previous problems associated with enzyme lability. Two methods, not directed to drug discovery, were utilized to stabilize PDF: addition of the enzyme catalase to the mixture containingg PDF or exchange of the natural iron metal catalytic center with another divalent metal like nickel (Ragusa et al., (1998) *J. Mol. Biol.* **280**:515-523). The addition of another enzyme, such as catalase, to stabilize PDF is problematic because this additional enzyme may interact with substrates and products or interfere with the assay thereby altering the results of experiments carried out with the addition of the stabilizing enzyme. (Groche et al., (1998) *Biochem. Biophys. Res. Comm.* **246**:342-346). The exchange of the iron metal center with nickel is not ideal since nickel and iron have different physiochemical properties. It would be preferable to use PDF with it's native iron catalytic metal center. It would be desirable to

have a stabilized form of PDF which avoids the abovementioned problems.

There is a need to develop new antimicrobial drugs in view of the capability of microbes to develop resistance to existing drugs. The use of antimicrobial drugs that are selective inhibitors of peptide deformylase is desirable in order to avoid deleterious side effects associated with therapeutic drugs that affect the host's biochemistry. In the case of peptide deformylase, it would be desirable to have drug discovery assays based on peptide deformylase inhibition that use the native iron catalytic center. In addition, it is most desirable that the peptide deformylase inhibitors discovered using these drug discovery assays be selective for peptide deformylase over other related enzymes such as angiotensin converting enzyme (ACE) thermolysin, collagenase and carboxypeptidase. Such selectivity minimizes the opportunity of adverse events associated with imbalances of these enzymes in the homostasis of tissue and organ functions affected by these metalloproteases.

3. SUMMARY OF THE INVENTION

The invention provides for novel drug discovery assays which specifically target peptide deformylase. The invention also provides for compounds detected by the assay and methods of making and using these compounds.

In one embodiment, the present invention provides a method of screening therapeutic compounds useful in the treatment of bacterial infections. The methods identify compounds that specifically target native bacterial peptide deformylase. The drug identification methods of the invention utilize peptide deformylase containing the typically unstable native catalytic iron metal center. In various embodiments, the invention provides a screening assay, to identify inhibitors of peptide deformylase, secondary *in vitro* PDF assays and lead compounds that score positive in these assays. The assays of the invention are adaptable to high-throughput screening.

In another embodiment, the invention provides compounds of the invention discovered by the screening methods described above. These compounds are capable of causing PDF inhibition in a bacterial cell, leading to a reduction or inhibition of bacterial growth. These compounds are expected to be effective against a variety of species of bacteria, including infectious pathogenic bacteria. The invention also includes novel pharmaceutical compositions which comprise compounds discovered as described below, formulated in pharmaceutically acceptable formulations.

Additionally, the invention includes pharmaceutical compositions and therapies comprising a compound of the invention and a second antibacterial compound including antibiotics of the following groups consisting of aminoglycosides, amphenicols, ansamycins, β-lactams, cephalosporins, cephamycins, monobactams, oxacephems, penicillins, lincosamides, macrolides, polypeptide antibiotics, tetracyclines, 2,4-diaminopyrimidines, nitrofurans, quinolones, streptogramins, sulfonamides, sulfones, oxazolidinones and glycylcyclines.

In yet another embodiment, the invention features a method for treating a subject infected with an infectious agent by administering to that subject a therapeutically effective amount of an antibiotic agent which causes PDF inhibition in the infectious agent as determined by the assays of the invention. Such administration can be by any method known to those skilled in the art, for example, by topical application or by systemic administration.

In another embodiment the compounds of the present invention, generally, have greater than or equal to 10-fold and preferably greater than or equal to 100-fold selectivity for PDF over metalloproteases like collagenase, thermolysin, angiotensin converting enzyme and carboxypeptidase.

A preferred embodiment of the invention involves the use of compounds or compositions of the invention against bacteria that are resistant to other antibiotics such as β-lactam, quinolone and vancinomycin resistant bacteria.

The invention provides for compounds that selectively inhibit peptide deformylase for the treatment of diseases caused by bacterial infections.

4. DETAILED DESCRIPTION OF THE INVENTION

The screening assays of the invention target peptide deformylase, which is unique to bacteria and essential for its growth. The assays can identify compounds that bind to bacterial peptide deformylase and affect its function. Deformylase of any prokaryotic origin can be used in the screening assays. The peptide deformylase of *E. coli* is preferred. In a highly preferred embodiment, the screening assays of the invention use peptide deformylase with its native catalytic iron metal. The invention utilizes stabilized iron peptide deformylase. The use of non-native metal centers, additional enzymes in the buffer system, or non-stabilized deformylase could complicate the interpretation of results obtained with their use. Previous assay systems relied on the addition of other enzymes or the use of a non-native catalytic metals to promote enzyme stability. However, the novel methods of the present invention avoid the use of certain extraneous factors such as non-native catalytic metals or additional enzymes, in the assay system to enhance enzyme stability.

The primary screening assay is designed to screen chemical compound libraries at high-throughput. Libraries containing single compounds, mixtures of compounds with known and unknown structures, and natural product extracts are all contemplated by the invention. Mixtures of compounds can be deconvoluted to identify the active compound. The active component(s) in natural product extracts that test positive in the assay are separated and identified. The high-throughput screening assay is an effective method for identifying compounds that interfere with peptide deformylase function. The follow-up assays confirm the mechanism of action of compounds that inhibit peptide deformylase function in the screening assay. Knowledge of their mechanism of

action facilitates the chemical optimization process and further structure-activity relationship studies to identify drug candidate compounds.

The selectivity assays are provided to evaluate hits identified in the primary screen. The selectivity assays identify compounds which have greater or equal to 10-fold, and preferably greater than or equal to 100-fold selectivity for peptide deformylase over ACE, thermolysin, collagenase and carboxy peptidase.

Such selectivity minimizes the opportunity in the infected host of adverse events such as imbalances in the homeostasis of a variety of tissue and organ functions. For example, an imbalance of collagenase in the extracellular matrix of the host, by inhibiting collagenase, for example, may lead to collagen accumulation. Collagen accumulation underlies a variety of degenerative and fibrotic diseases, including pulmonary fibrosis, liver cirrhosis and retrocorneal fibrous membrane formation. Likewise, carboxypeptidases are found in pancreatic tissue and are involved in the digestion of peptides. Angiotensin converting enzyme increases arterial impedance when physiological conditions demand.

Follow up assays are provided which are designed to further characterize the mode of action of inhibitors on peptide deformylase function. Compounds identified in the assay based on growth inhibition of a test species, such as *E. coli*, and *Staphylococcus aureus*, are tested for efficacy against other bacterial pathogens to confirm that the positive compounds are effective against a broad range of microorganisms. Furthermore, these compounds are tested for toxicity in mammalian cells to confirm the bacterial selectivity of the target.

The methods of the invention have a high probability of identifying useful drugs for several reasons. First, the screening system targets an essential factor that is ubiquitous and conserved throughout the bacterial kingdom. The peptide deformylase target is highly selective as no

homologous mammalian counterpart exists. The high-throughput primary screen of the invention allows for easy visible identification of positive hits. Further, the selectivity assays provide evidence that the compounds identified by the screening system are selective for the peptide deformylase target over several closely related enzymes as detailed below.

4.1 Assays and Selectivity Profile

Peptide deformylase is an extremely labile enzyme, with a half-life on the order of minutes. This instability has made PDF a difficult enzyme to characterize. Several approaches, by other researchers, were followed to circumvent this instability problem. For example, other metal ions, such as nickel, have been substituted for the catalytic iron metal in PDF (Groche et al.,(1998) *Biochem. Biophys. Res. Comm.* **246**:342-346). The applicability of results obtained with studies conducted using the non-native metal center remains in question. Another attempt to stabilize PDF utilized the enzyme catalase in the assay buffer solution (*Id.*). A caveat to the catalase based stabilization of PDF is that additional enzyme components in the assay buffer systems complicate the interpretation of results obtained using such a modified system. During the studies with catalase it was found that oxygen exclusion methods, such as running the deformylase reaction under argon, were ineffective in enhancing PDF stability. In a similar report, Rajagopalan and Pei developed a method of enzymatically excluding oxygen from the PDF containing solutions that greatly improved PDF stability (1998, *J. Biol. Chem.* **273**:22305-22310). The exclusion of oxygen in the solutions containing PDF was accomplished through the addition of the enzymes catalase and glucose oxidase, and glucose.

It has been discovered by the inventors of the present invention that PDF activity can be stabilized by two novel and complementary methods that are amenable to drug discovery paradigms. The primary novel drug discovery assay of the

invention relies on the use of *E. coli* lysates to detect compounds that inhibit PDF. It was discovered by the inventors that PDF containing lysates had enhanced stability if the solutions containing the lysates were manipulated in a manner which reduces the introduction of oxygen. The assays of the invention allow for the rapid screening of compounds that inhibit PDF in native lysates with stabilized PDF.

The secondary assays of the invention involve the kinetic characterization of inhibitors and thus requires PDF with a greater level of purity than provided in crude lysates. The drug screening assays of the invention utilize tris(carboxyethyl) phosphine, TCEP, as the buffering agent, which stabilizes PDF. These complementary methods of stabilizing PDF allow for the use of PDF with its native iron catalytic metal center in the assays of the invention.

The present methods of stabilizing PDF are preferable to other methods in the literature for several reasons. One, they are relatively non-invasive techniques, thus results obtained with such an assay system are more reliable, relevant to *in vivo* situations and more applicable to drug discovery schemes. Two, they are complementary within the context of drug discovery. The primary screens use native PDF from *E. coli* lysates. The use of crude lysates in the primary screen to detect PDF inhibition is a superior indicator of potential antimicrobial activity because in the lysate the PDF is in its native biochemical context. The secondary selectivity assays are used to determine kinetic parameters of PDF inhibition to determine the selectivity profile of the inhibitor. The secondary assays require PDF in a controlled context: purified, stabilized, of given concentration and activity. This is accomplished in the invention through the use of PDF in solutions containing TCEP. The use of these two methods in combination with the secondary selectivity assays provide the means for rapid screening, discovery and development of antimicrobials that selectively inhibit PDF.

Primary Screen

The invention provides a primary screen that uses native *E. coli* peptide deformylase. The primary screen for determination of peptide deformylase inhibition involves the incubation of a formylated peptide substrate, formyl-methionine-alanine-serine-OH (fMAS), with an *E. coli* BL21 lysate containing both deformylase and aminopeptidase, along with the compound(s) to be tested. It is understood that any bacterial strain having normal peptide deformylase activity can be used to form the lysate. Peptide deformylase is an enzyme which removes the formyl group from the substrate peptide. Peptide deformylase, by removing the formyl group, deprotects the fMAS substrate, thus allowing for further processing by aminopeptidase. Aminopeptidase cleaves the deformylated peptide into the three amino acids only if the fMAS peptide is deformylated. After an appropriate time interval, a ninhydrin reagent is added to the reaction mixture to detect the amount of free amino groups and allows for quantification of the deformylation reaction progress by measuring the absorbance at about 570 nanometers. A decrease in the ninhydrin signal as compared to the control indicates that either deformylase or aminopeptidase was disrupted by the test compound.

Compounds are generally tested in the primary screen at 0.1-100 μg/ml. It is expected that one skilled in the art will adjust the concentration to best suit ones needs. Preferably, the compounds are screened at 2-10 μg/ml. The positive control is a metal chelator which sequesters divalent metal ions in the reactions. A preferred positive control is 1,10 phenanthroline. Freshly thawed *E. coli* BL21 lysate (E. coli lysate S30, Promega Inc., Madison, WI, is preferred), containing native *E. coli* peptide deformylase and aminopeptidase is diluted 1:3 in a tris-acetate buffer system so as to avoid bubbling. All manipulations are performed carefully to avoid the introduction of air bubbles into the lysate. The lysate is stable for only 30-45 minutes at room temperature or on ice and must be used immediately after

dilution. Diluted *E. coli* lysate is added to each test well, including the control wells. The reactions are not mixed at this stage to avoid bubbling. The fMAS substrate is slowly injected into the reactions. At this point the plate of reaction mixtures should have few or no bubbles.

Test plates containing the reaction mixtures are incubated for approximately 1 hour at 30 °C in a water bath. The type and size of the test plate can be varied in accordance with ones needs. Polypropylene plates having 96- or 384-wells are preferred. The 1 hour incubation is followed by addition of the ninhydrin reagent. Next the plates are covered and vortexed on low for approximately 10 seconds and incubated for about 20 minutes at 45 °C in a water bath. The optical density at 560-580 nanometers, and preferably 570 nanometers is determined in a spectrophotometer. Background wells contain diluted *E. coli* lysate, water and 0.8% DMSO. The positive control wells, *i.e.*, phenanthroline inhibitor wells, contain phenanthroline, lysate and fMAS. The "no compound" wells (negative wells) contain 0.8% DMSO, lysate and fMAS.

Selectivity Profile Assays

Compounds identified in the initial screen are used to determine their binding affinity to PDF in a novel assay that utilizes the enzyme's native iron metal center and the binding affinity to several other metalloproteases including thermolysin, carboxypeptidase, collagenase and angiotensin coverting enzyme. The selectivity profile is then determined by calculating the ratio of the binding affinity of the inhibitor to: (1) peptide deformylase with it's native iron metal center and (2) other metalloproteases. It is preferred that compounds bind to PDF with a greater than or equal to 10-fold affinity relative to the other metalloproteases. It is highly preferred that compounds bind to PDF with a greater than or equal to 100-fold affinity relative to the other metalloproteases. In another embodiment, it is preferred that compounds bind to PDF with a greater than or equal to

100-fold affinity relative to thermolysin and greater than or equal to 300-fold relative to carboxypetidase, collagenase and angiotensin converting enzyme. The methods of the invention are a substantial improvement over prior technologies in that: the inhibition of peptide deformylase is determined using the enzyme's native iron catalytic metal center and the risk of deleterious side effects can be minimized since the novel inhibitors identified by the means of the invention interact negligibly with important metalloproteases of the host's biochemical machinery. The metalloproteases that are tested include thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

Initial assays are performed to determine the percent Fe-PDF inhibition at a particular concentration of test compound. The concentration of test compound is typical between 1 μM - 5 mM. While the concentration may be varied, a preferred concentration of test compounds is 300 μM. The concentration of reagents described below reflects assays which contain 300 μM of test compound. However, the concentration of reagents can be easily adjusted to accommodate the concentration of test compound given the parameters provided by the invention in the disclosure below. The reactions are set up in polypropylene plates. Preferred plates have 96- or 384-wells. A preferred plate is a 96-well polypropylene plate with 12 columns and 8 rows. One column is reserved for the negative control (20% DMSO), one column is for the positive control (actinonin, Sigma, St. Louis, MO) and the rest are for test compounds. The reaction mixture, containing CHELEX™ 100 (Sigma, St. Louis, MO) treated water, 10.6 mM NaCl, 1.06 mg/ml BSA, 10.6 mM TCEP-HCl (Pierce, Rockford Il.) 35.3 mM fMAS, 23.5 mM NAD and 17.6 U/ml formate dehydrogenase (FDH) (Roche Molecular Biochemicals, Indianapolis, IN) is agitated and approximately 85 μl of the reaction mixture is then added to each well on the reaction plate. Endpoint data is recorded in a spectrophotometer by monitoring at 335-345 nanometers. 340 nanometers is

preferred. To initiate the reactions 10 μl of 1/20,000 fold diluted Fe-PDF (Rajagopalan et al., (1997) *J. Am. Chem. Soc.* 119:12418-12419; Rajagopalan et al., (1997) *Biochemistry* 36:13910-13918, both of which are incorporated by reference) is added to each well. The TCEP buffer is necessary for stabilizing the diluted enzyme, see section 5.2. The reaction plate is then mixed on a vortex mixer at low speed. Next the reactions are monitored in a spectrophotometer. A preferred spectrophotometer is the Spectromax 250 (Molecular Devices Corp., Sunnyvale, CA). Analysis of results from these experiments allows the determination of percent inhibition of Fe-PDF at 300 μM test compound.

The assays to determine the binding affinity of the test compounds are based on the results obtained from the percent Fe-PDF inhibition studies. The initial concentrations of the test compounds in the binding affinity assays are adjusted according to the results in the percent Fe-PDF inhibition studies.

Compounds are initially prepared in a 96-well working plate with 12 columns and 8 rows. Again, the invention is not limited to this size plate, one skilled in the art can choose one appropriate to ones needs. One column is for the negative control (20% DMSO), another is reserved for the serial dilutions of the positive control, actinonin, and the rest are for the test compounds. The test compounds are diluted from 1/5 to 1/640 fold. 85 μL of reaction mixture is added to each well along with 5 μL of the appropriately diluted test compound. The reactions are initiated by addition of 10 μL of 1/20,000 fold dilute Fe-PDF. After addition of the Fe-PDF the plate is immediately inserted into the spectrophotometer and monitored at 335-345 nanometers and preferably 340 nanometers. Analysis of the data provided from the spectrophotometer readings gives the binding affinities for the test compounds.

The thermolysin assay is designed to detect compounds that have an inhibitory effect on the metalloprotease thermolysin. The assay is based on the use of succinylated

casein in conjunction with TNBSA (trinitrobenzene sulfonic acid)(Hatakeyama et al., (1992) *Anal. Biochem.* **204**:181-184; Bubnis et al., (1992) *Anal. Biochem.* **207**:129-133; Habeeb et al., (1996) *Anal. Biochem* **14**:328-336). Native casein has been treated with succinic anhydride to block available primary amines on the surface of the protein. Thermolysin acts to cleave the peptide bonds to expose primary amines. TNBSA reacts with primary amines to produce a yellow-orange color that can be detected at A430 and quantitated. A control well consisting of substrate, enzyme, and TNBSA with 1% DMSO is used to determine the basal signal level. Any test well with a relative signal lower than the control level would indicate that thermolysin activity has been disrupted. The known inhibitor of thermolysin, phosphoramidon, is added for the calculation IC50 data (*i.e.*, the concentration of test compound that results in a fifty percent reduction of enzyme activity).

The carboxypeptidase A assay is designed to detect compounds that have an inhibitory effect on Carboxypeptidase A. The assay is based on the use of a blue shift of the absorption spectrum that occurs upon the hydrolysis of a furanacryloyl peptide FAPP (FA-Phe-Phe-OH) by the enzyme (Peterson et al., (1982) *Anal. Biochem.* **125**:420-426, which is incorporated by reference in its entirety). Activity by the enzyme will produce a decrease in OD at A330 between an initial reading at time zero and a second reading after one hour. A control-well consisting of enzyme, substrate, and 1% DMSO is used to determine a maximum OD. Any test well with an OD lower than the control well will indicate that the enzyme activity has been disrupted. A known inhibitor of Carboxypeptidase A from potato tubers is added for the calculation of IC50 data.

The collagenase assay is designed to detect compounds that have an inhibitory effect on bacterial collagenase. The assay is based on the use of a blue shift of the absorption spectrum that occurs upon the hydrolysis of a furanacryloyl peptide FALGPA(FA-Leu-Gly-Pro-Ala-OH) by the enzyme (Van Wart

et al., (1981) *Anal. Biochem.* **113**:356-365, which is incorporated by reference in its entirety). Activity by the enzyme will produce a decrease in OD at A330 between an initial reading at time zero and a second reading after twenty minutes. A control-well consisting of enzyme, substrate, and 1% DMSO is used to determine a maximum OD. Any test well with a OD lower than the control well would indicate that the enzyme activity has been disrupted. A peptide that is a known inhibitor of collagenase is used for calculation if IC50's.

The angiotensin converting enzyme (ACE) assay is designed to detect compounds that have an inhibitory effect on the enzyme ACE. Angiotensin converting enzyme is a halide-activated peptidase that splits off hydrolytically the dipeptide His-Leu from the carboxyl end of the decapeptide angiotensin I (Asp-Arg-Val-Try-Ile-His-Pro-Phe-His-Leu), thereby converting it to the vasopressor octapeptide angiotensin II. The assay utilizes the internally quenched fluorescent tripeptide derivative Abz-Gly-Phe(NO_2)-Pro. The fluorescent aminobenzoyl (Abz) group incorporated in this tripeptide can be excited in the 300-380 nm range upon cleavage of the peptide bond Gly-Phe(NO_2)-Pro (Carmel and Yaron, (1978) *Anal. Biochem.* **87**:265-273, which is incorporated by reference in its entirety). Fluorescence is proportional to the amount of liberated Abz-Gly, and a percent control value can be determined by comparing the fluorescence of a sample compound well to that of a control well containing only 1% DMSO. The substrate has a published K_m = 0.21 mM ± 0.1 mM.

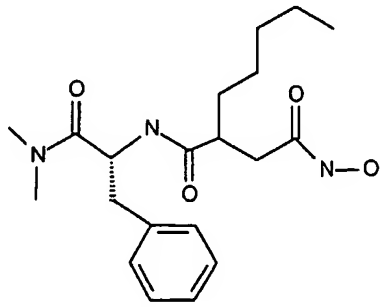
During the course of screens designed to identify inhibitors of peptide deformylase, it was discovered by the present inventors that a variety of compounds inhibit peptide deformylase with a greater than or equal to 10-fold affinity, and preferably a greater than or equal to 100-fold affinity, relative to other metalloproteases.

4.2 COMPOUNDS IDENTIFIED BY METHODS OF THE INVENTION

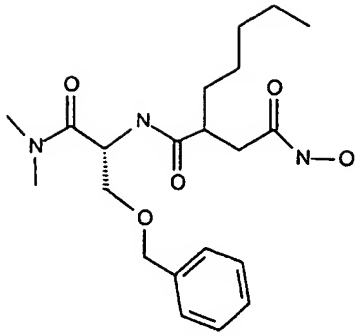
Using the novel screening methods described above, the inventors have discovered that certain metalloprotease inhibitors, identified for purposes other than antibacterial chemotherapeutics, inhibit peptide deformylase.

Several of the compounds of the invention were previously described as matrix metalloprotease inhibitors. Compound 1 and Compound 2 and their synthesis are described in United States Patent No. 5,902,791 as matrix metalloprotease inhibitors. United States Patent No. 5,902,791 is incorporated by reference in its entirety for all purposes. Two other structurally related compounds, Compound 3 and Compound 4, were described in WO99/44989 (which is incorporated by reference in its entirety for all purposes) as matrix metalloprotease inhibitors. Compound 1, Compound 2, Compound 3 and Compound 4 were described as having potential applications in the treatment of diseases characterized by disorders in connective tissue breakdown that are mediated by matrix metalloproteases. These previously identified metalloprotease inhibitors were described for the potential prophylaxis or treatment of diseases that include tumor metastasis, invasion and growth, rheumatoid arthritis, neuroinflammatory disorders and angiogenesis dependent disorders.

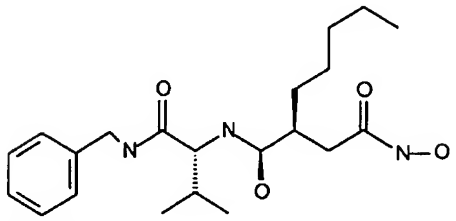
However, the present inventors discovered that these matrix metalloprotease inhibitors, whose structures are given below and which have been explored only for the treatment of diseases related to connective tissue disorders, are potent peptide deformylase inhibitors with antibacterial activity:



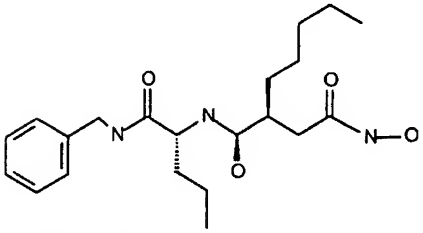
Compound 1



Compound 2

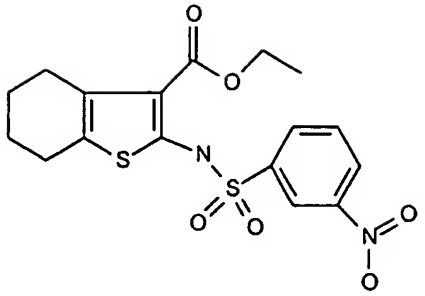


Compound 3

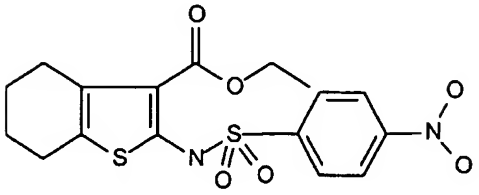


Compound 4

The preparation and use of compounds with structures similar to Compound 5 as herbicides was described in United States Patent No. 5,480,857 which is incorporated by reference in its entirety for all purposes. The synthesis of a structurally related compound Compound 6 was reported in Shvedov et al., (*Chem. Heterocycl. Compd. (Engl. Trans.)* (1977) **13**:163). Compound 5 and Compound 6 were not previously known to have antibacterial activity.



Compound 5



Compound 6

The present inventors have discovered that these compounds are selective inhibitors of peptide deformylase. In particular, they have an approximately one hundred fold binding preference for PDF as compared to thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

Further, it was discovered by the inventors that certain hydroxamic acid derivatives selectively inhibit PDF. These hydroxamic acid derivatives bind PDF with a greater affinity than they bind to other metalloproteases.

The hydroxamic acid antibiotic, actinonin, the positive control in the assays of the invention, was first described in 1962 (Gordon et al., (1962) *Nature* **195**:701) and was covered by United States Patent 3,240,787 (1966). Actinonin is a compound produced by the bacterium Streptomyces actinomycete. Actinonin has the following general structure:

$$R_1CO\text{-}CH(R_2)\text{-}NH\text{-}CO\text{-}CH(R_3)\text{-}CH_2\text{-}CO\text{-}NH\text{-}OH$$

where R_1 is l-prolinol, R_2 is iso-propyl and R_3 is n-pentyl.

The invention provides for novel compounds that have antibacterial activity and selectively bind PDF with the following structure:

$$R_1CO\text{-}CH(R_2)\text{-}NH\text{-}CO\text{-}CH(R_3)\text{-}CH_2\text{-}CO\text{-}NH\text{-}OH$$

where R_1 is a cyclic amino group;

R_2 is hydrogen, C_1-C_6 alkyl, alkenyl, aryl, heterocyclic;

R_3 is hydrogen, C_1-C_6 alkyl, alkenyl, aryl, heterocyclic;

where the compound binds PDF with a greater affinity than it binds to other metalloproteases with the provisio that the compound does not have the following structures:

a) R_1 is morpholine, R_2 is isopropyl and R_3 is selected from the group consisting of methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, iso-hexyl, CH_2CH, Ph, $PhCH_2$, p-$ClC_6H_4CH_2$; or

b) R_1 is morpholine, R_2 is either iso-butyl, methyl or hydrogen and R_3 is n-pentyl.

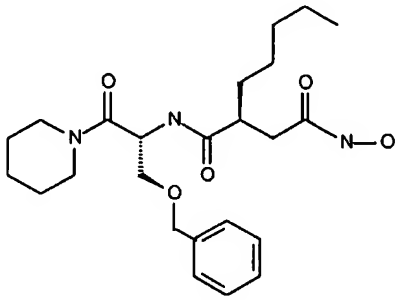
As used herein the term C_1-C_6 alkyl means a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms including but not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl.

As used herein the term cycloalkyl means a saturated alicyclic moiety having from 3-8 carbons including but not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

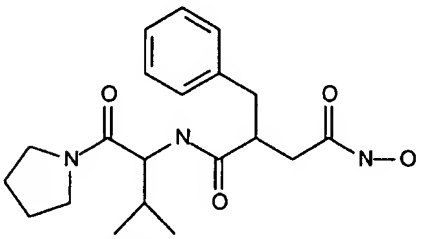
As used herein the term heterocyclyl or heterocyclic means a 5-7 member ring, either aromatic or non-aromatic, containing one or more heteroatoms selected from nitrogen, sulfur, and oxygen, and optionally fused to a benzene ring system examples include but are not limited to thiazol,

imidazolyl, oxazoly, indoyl, morhpholinyl, pyridinyl, pyrrilidinyl, pyrimidinyl, thienyl, furyl, piperidinyl, piperazinyl.

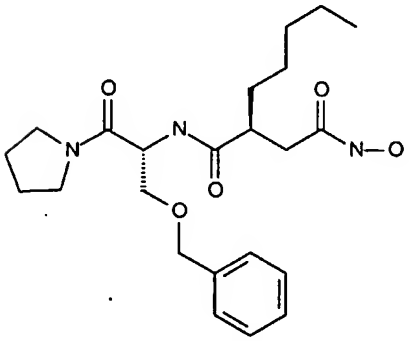
Preferred compounds have the following structures:



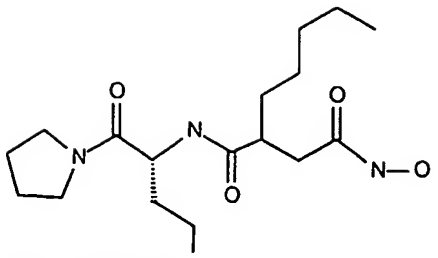
Compound 7



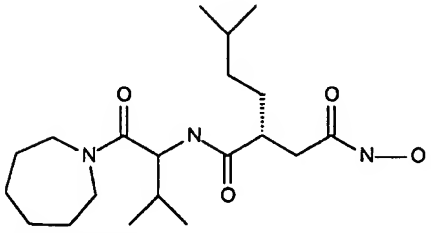
Compound 8



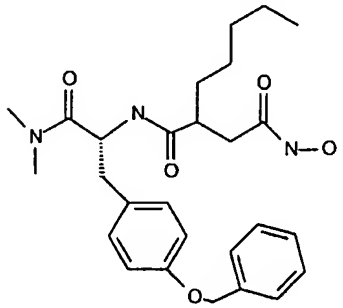
Compound 9



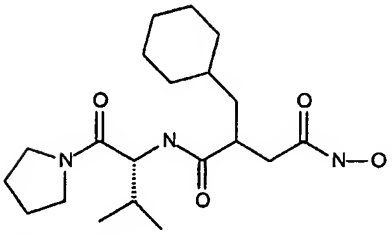
Compound 10



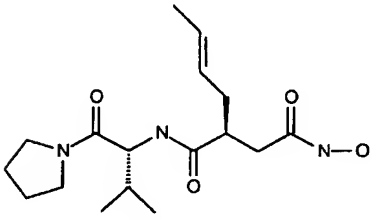
Compound 11



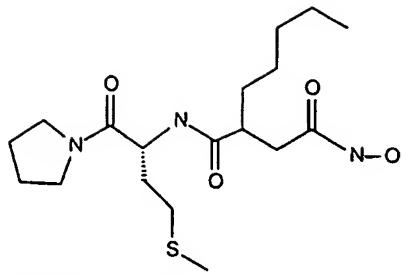
Compound 12



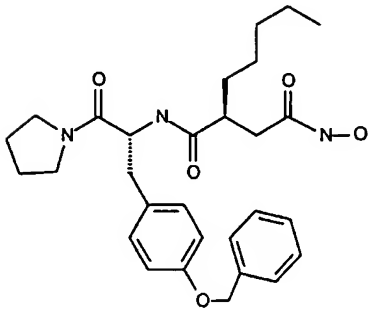
Compound 13



Compound 14

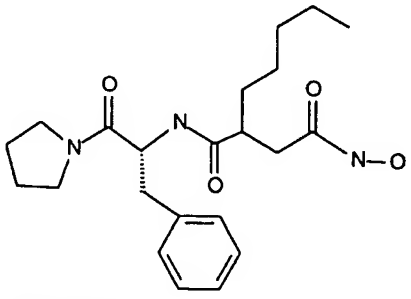


Compound 15

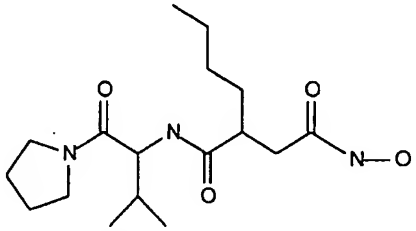


Compound 16

The inventors have discovered that the following two compounds have, in addition to antibacterial activity, a binding preference for PDF as compared to the metalloproteases thermolysin, carboxypetidase, collagenase and angiotensin converting enzyme.



Compound 17



Compound 18

There are several actual or potential chiral centers in the compounds according to the invention due to the presence of asymmetric carbons. More than one chiral carbon within a compound gives rise to stereoisomers with R or S stereochemistry at each chiral center. The invention includes all such stereoisomers, diastereomers, enantiomers, and all racemic or optically pure forms of the compounds and their metabolites.

The compounds of the invention are expected to be effective against a variety of species of bacteria, including infectious pathogenic bacteria. The invention also includes novel pharmaceutical compositions which comprise the compounds of the invention formulated in pharmaceutically acceptable formulations.

In yet another embodiment, the invention features a method for treating a subject infected with an infectious agent by administering to that subject a therapeutically effective amount of a compound of the invention which causes PDF inhibition in the infectious agent as determined by the assays of the invention. Veterinary uses are also included in this embodiment, as described below. Such administration can be by any method known to those skilled in the art, for example, by topical application or by systemic administration. Additional antibacterial compounds may be adjunctively administered, as described below.

In yet another embodiment, compounds of the invention of the present invention can be used to treat contaminated items, such as crops, wood, metal or plastic and the like, by methods such as, but not limited to, spraying or dusting of that agent onto the contaminated item, or impregnating that agent into the item.

By "therapeutically effective amount" is meant an amount that relieves (to some extent) one or more symptoms of the disease or condition in the subject. Additionally, by "therapeutically effective amount" is meant an amount that returns to normal, either partially or completely,

physiological or biochemical parameters associated with or causative of a bacterial disease or condition.

Direct Binding Assays

Direct binding of a test compound to PDF may also be tested in the screening assays of the invention. Purified PDF is preferred for these assays. PDF protein may be enriched and purified by a variety of methods known in the art. In one embodiment, PDF may be purified from bacterial cell extracts, for example, by antibody affinity purification. In another embodiment, PDF may be produced by recombinant means. For example, *in vitro* translation methods or induction of expression from a bacterial expression vector containing the PDF open reading frame may be used. The PDF protein may be tagged with a peptide sequence that facilitates its purification and its attachment to a solid phase, for example glutathione-S-transferase (GST) or poly-histidine (p-His).

In a direct binding assay, the PDF protein is contacted with a test compound under conditions that allow binding of the test compound. The binding may take place in solution or on a solid surface. Preferably, the test compound is previously labeled for detection. Any detectable compound may be used for labeling, such as but not limited to, a luminescent, fluorescent, or radioactive isotope or group containing same, or a nonisotopic label, such as an enzyme or dye. After a period of incubation sufficient for binding to take place, the reaction is exposed to conditions and manipulations that remove excess or non-specifically bound test compound. Typically, it involves washing with an appropriate buffer, Finally, the presence of an PDF-test compound complex is detected.

Scintillation Proximity Assay

In one embodiment of the invention, direct binding may be performed using a scintillation proximity assay (described in U.S. patent no. 4,568,649 which is incorporated

by reference in its entirety). Purified PDF is coated onto the surface of a scintillant-loaded solid phase (e.g., beads) and the solid phase are treated with a blocking agent such as albumin or serum. Radiolabeled test compounds (e.g., 33P-labeled) are then mixed with the PDF coated beads, under conditions that would allow specific binding of a candidate specific binding test compound to the PDF on the solid phase. After washing to remove excess or non-specific binding, if specific binding of a labeled test compound and PDF took place, the radiolabel is brought into close proximity to the scintillant, allowing the scintillant to emit light, which is detectable with a scintillation counter.

In an alternative embodiment, an affinity capture scintillation proximity assay may be used so that binding may be performed in solution. In this assay, PDF is purified and labeled with an affinity label, such as biotin. Biotinylated PDF is then mixed with the radiolabeled test compound, under conditions that allow solution binding to occur. Biotinylated PDF including complexes of PDF and test compound are captured on streptavidin-coated scintillant-loaded beads (available from Amersham) and counted in a scintillation counter, as described above.

Binding on a Solid Surface

In another embodiment, an affinity binding assay may be performed using a purified PDF which is immobilized to a solid support. In various embodiments, the solid support could be, but is not restricted to, polycarbonate, polystyrene, polypropylene, polyethlene, glass, nitrocellulose, dextran, nylon, polyacrylamide and agarose. The support configuration can include beads, membranes, microparticles, the interior surface of a reaction vessel such as a microtiter plate, test tube or other reaction vessel. The immobilization of PDF can be achieved through covalent or non-covalent attachments. In one embodiment, the attachment may be indirect, *i.e.*, through an attached antibody. In another embodiment, PDF and negative controls

are tagged with an epitope, such as glutatione S-transferase (GST) so that the attachment to the solid surface can be mediated by a commercially available antibody such as anti-GST (Santa Cruz Biotechnology).

The test compound is labeled, to enable detection. A variety of labeling methods are available and may be used, such as luminescent, chromophore, fluorescent, or radioactive isotope or group containing same, and nonisotopic labels, such as enzymes or dyes. In a preferred embodiment, the test compound is labeled with a fluorophore such as fluorescein isothiocyanate (FITC, available from Sigma Chemicals, St. Louis).

The labeled test compound is then added to the surface and allowed to bind. After the binding reaction has taken place, unbound and non-specifically bound test compound is removed by means of washing the surface, and the remaining label may be detected by any detection method known in the art. For example, if the test compound is labeled with a fluorophore, a fluorimeter may be used to detect complexes.

Determination of MIC

The minimum inhibitory concentration (MIC) against bacterial organisms is determined for each compound that is positive in both the primary screens and selectivity assays (as described in section 4.1). Methods known in the art may be used such as broth microdilution testing, using a range of concentrations of each test compound (1993, National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests For Bacteria That Grow Aerobically - Third Edition: Approved Standard, M7-A3, which is incorporated by reference herein in its entirety). The MIC against a variety of pathogens are determined using the same method. Pathogenic species to be tested generally include, but are not limited to: *E. coli*, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas*

aeruginosa, *Staphylococcus epidermis*, *Shigella flexneri*, and *Salmonella typhimurium*.

Cytotoxicity Testing

Unfortunately, toxicity does not always arise from the same mechanism of action as is responsible for growth inhibition in the targeted microorganism. Therefore, the selectivity of the target should not be assessed solely on the basis of these results.

Cytotoxicity can be measured by methods known in the art. One such method is based on assessing growth of mammalian cells in the presence of the test compound. The assay measures the metabolic reduction by viable cells, of colorless XTT terazolium to yield orange XTT formazan, which is measurable by conventional colorimetric techniques (Weislow et al., 1989, *J. Natl. Cancer Inst.* 81:577-586; which is incorporated by reference in its entirety). It is to be understood that the present invention has application for both human and veterinary use.

Formulation

The compounds described above can be provided as pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. Administration as used in the invention includes those suitable for oral, ophthalmic, (including intravitreal or intracameral), topical, mucosal (including buccal, rectal, vaginal, nasal and sublingual), transdermal or parenteral (including subcutaneous, intramuscular, intravenous, bolus injection, intradermal, intratracheal, and epidural) administration. In addition, the combinations may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired. Biodegradable polymers and their use are described, for example, in detail in Brem *et al.*, *J. Neurosurg.* 74:441-446 (1991).

The formulations include those suitable for oral, rectal, ophthalmic, (including intravitreal or intracameral) nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into associate the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tamports, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present invention may include other agents conventional in the art having regard to the type of

formulation in question, for example, those suitable for oral administration may include flavoring agents.

Oral dosage forms include tablets, capsules, dragees, and similar shaped, compressed pharmaceutical forms containing from about 1 ng to 500 mg of drug per unit dosage. Isotonic saline solutions can be used for parenteral administration which includes intramuscular, intrathecal, intravenous and intra-arterial routes of administration. Rectal administration can be effected through the use of suppositories formulated from conventional carriers such as cocoa butter.

Pharmaceutical compositions thus comprise one or more compounds described above and are associated with at least one pharmaceutically acceptable carrier, diluent or excipient. In preparing such compositions, the active ingredients are usually mixed with or diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule or sachet. When the excipient serves as a diluent, it may be a solid, semi-solid, or liquid material which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders. Examples of suitable excipients, include but are not limited to lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidinone, cellulose, water, syrup, and methyl cellulose, the formulations can additionally include lubricating agents such as talc, magnesium stearate and mineral oil, wetting agents, emulsifying and suspending agents, preserving agents such as methyl- and propylhydroxybenzoates, sweetening agents or flavoring agents.

The compositions preferably are formulated in unit dosage form, meaning physically discrete units suitable as a unitary dosage, or a predetermined fraction of a unitary dose

to be administered in a single or multiple dosage regimen to human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with a suitable pharmaceutical excipient. The compositions can be formulated so as to provide an immediate, sustained or delayed release of active ingredient after administration to the patient by employing procedures well known in the art.

The pharmaceutical compositions of the present invention comprise an antibiotic compound as the active ingredient, or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier, and optionally, other therapeutic ingredients, for example antivirals. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic acids and bases, including inorganic and organic acids and bases.

The compounds of the invention that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. Acids that can be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds of the invention are those that form non-toxic acid addition salts, *i.e.*, salts containing pharmacologically acceptable anions, such as, but not limited to, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, formate, acetate, propionate, succinate, camphorsulfonate, citrate, acid citrate, fumarate, gluconate, isethionate, lactate, malate, mucate, gentisate, isonicotinate, saccharate, tartrate, bitartrate, para-toluenesulfonate, glycolate, glucuronate, maleate, furoate, glutamate, ascorbate, benzoate, anthranilate, salicylate, phenylacetate, mandelate, embonate (pamoate), methanesulfonate, ethanesulfonate, pantothenate, benzenesulfonate, stearate, sulfanilate, alginate, p-toluenesulfonate, and galacturonate. Particularly preferred anions are hydrobromide, hydrochloride, phosphate, acid phosphate, maleate, sulfate,

and acid phosphate. Most particularly preferred anions are hydrochloride and maleate.

Compounds of the invention that are acidic in nature are capable of forming salts with various pharmaceutically acceptable bases. The bases that can be used to prepare pharmaceutically acceptable base addition salts of such acidic compounds of the invention are those that form non-toxic base addition salts, *i.e.*, salts containing pharmacologically acceptable cations such as, but not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumaine (N-methylglucamine), lysine, and procaine.

As used herein, the terms "avoiding adverse side effects" and "avoiding adverse effects" mean eliminating or reducing at least one adverse effect associated with the administration of a particular compound or mixture of compounds.

Administration

For administration to subjects, antibiotic compounds of the invention may be formulated in pharmaceutically acceptable compositions. The compositions can be used alone or in combination with one another, or in combination with other therapeutic or diagnostic agents. These compositions can be utilized *in vivo*, ordinarily in a mammal, preferably in a human, or *in vitro*. In employing them *in vivo*, the compositions can be administered to the mammal in a variety of ways, including parenterally, intravenously, subcutaneously, intramuscularly, colonially, rectally, vaginally, nasally, orally, transdermally, topically, ocularly, or intraperitoneally.

As will be readily apparent to one skilled in the art, the magnitude of a therapeutic dose of an antibiotic compound in the acute or chronic management of an infectious

disease will vary with the severity of the condition to be treated, the particular composition employed, and the route of administration. The dose and dose frequency will also vary according to the species of animal, age, body weight, condition and response of the individual subject. Such dosing schemes can be readily selected by those skilled in the art with due consideration of such factors.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid pharmaceutical carriers are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out above, an active ingredient can also be administered by controlled release means or delivery devices that are well known to those of ordinary skill in the art, such as those described in U.S. Patent Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,566, the disclosures of which are incorporated herein by reference. These dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an

optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include: 1) extended activity of the drug; 2) reduced dosage frequency; and 3) increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and thus can affect the occurrence of side effects.

Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various inducers, including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

Pharmaceutical compositions of the invention suitable for oral administration can be presented as discrete dosage forms, such as capsules, cachets, or tablets, or aerosol sprays each containing a predetermined amount of an active ingredient as a powder or in granules, a solution, or a suspension in an aqueous or non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. Such dosage forms can be prepared by any of the methods of pharmacy, but all methods include the step of bringing the active ingredient into association with the carrier, which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

For example, a tablet can be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets can be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granules, optionally mixed with an excipient such as, but not limited to, a binder, a lubricant, an inert diluent, and/or a surface active or dispersing agent. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms which comprises an active ingredient, since water can facilitate the degradation of some compounds. For example, the addition of water (*e.g.*, 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. *See, e.g.*, Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, NY, 1995, pp. 379-80. In effect, water and heat accelerate decomposition. Thus the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms of racemic or optically pure bupropion metabolite which contain lactose are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure

to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastic or the like, unit dose containers, blister packs, and strip packs.

In this regard, the invention encompasses a method of preparing a solid pharmaceutical formulation which comprises an active ingredient which method comprises admixing under anhydrous or low moisture/humidity conditions the active ingredient and an excipient (*e.g.*, lactose), wherein the ingredients are substantially free of water. The method can further comprise packaging the anhydrous or non-hygroscopic solid formulation under low moisture conditions. By using such conditions, the risk of contact with water is reduced and the degradation of the active ingredient can be prevented or substantially reduced.

Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (*e.g.*, ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (*e.g.*, Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Suitable forms of microcrystalline cellulose include, for example, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, and AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, PA, U.S.A.). An exemplary suitable binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Examples of suitable fillers for use in the pharmaceutical compositions and dosage forms disclosed herein

include, but are not limited to, talc, calcium carbonate (*e.g.*, granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder/filler in pharmaceutical compositions of the present invention is typically present in about 50 to about 99 weight percent of the pharmaceutical composition.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Too much of a disintegrant will produce tablets which may disintegrate in the bottle. Too little may be insufficient for disintegration to occur and may thus alter the rate and extent of release of the active ingredient(s) from the dosage form. Thus, a sufficient amount of disintegrant that is neither too little nor too much to detrimentally alter the release of the active ingredient(s) should be used to form the dosage forms of the compounds disclosed herein. The amount of disintegrant used varies based upon the type of formulation and mode of administration, and is readily discernible to those of ordinary skill in the art. Typically, about 0.5 to about 15 weight percent of disintegrant, preferably about 1 to about 5 weight percent of disintegrant, can be used in the pharmaceutical composition.

Disintegrants that can be used to form pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums or mixtures thereof.

Lubricants which can be used to form pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium

lauryl sulfate, talc, hydrogenated vegetable oil (*e.g.*, peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, or mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, MD), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Texas), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass), or mixtures thereof. A lubricant can optionally be added, typically in an amount of less than about 1 weight percent of the pharmaceutical composition.

The methods of the invention also encompass combination therapy in which an antimicrobial compound of the invention or identified by means of the invention is administered as an admixture or sequentially with at least one other antimicrobial. The second antibacterial compound may be naturally occurring or synthetic. Suitable naturally occurring antibacterial compounds include, but are not limited to, aminoglycosides (including but not limited to dihydrostreptomycin, gentamycin, kanamycin, neomycin, paromycin and streptomycin); amphenicols (including but not limited to chloramphenicol); ansamycins (including but not limited to rifamycin); β-lactams such as carbapems (including but not limited to imipenem), cephalosporins (including but not limited to cefazedone and cefroxadine), cephamycins (including but not limited to cefbuperazone); monobactams (including but not limited to aztreonam), oxacephems (including but not limited to flomoxef) or penicillins (including but not limited to ampicillin, carbencillin, methicillin, penicillin N, penicillin O and penicillin V); lincosamides (including but not limited to clindamycin and lincomycin); macrolides (including but not limited to carbomycin and erythromycin); polypeptides (including but not limited to gramicidin S and vancomycin); tetracyclines (including but not limited to apicycline, methacycline and tetracycline); and others such as cycloserine, mupirocin and

tuberin. Suitable synthetic antibacterial compounds include 2,4-diaminopyrimidines (including but not limited to trimethoprim); nitrofurans (including but not limited to nifuradene); quinolones and quinolone analogs (including but not limited to enoxacin, lomefloxacin, nalidixic acid and ofloxacin); streptogramins; sulfonamides (including but not limited to sulfamoxole and sulfanilamide); sulfones (including but not limited to diathymosulfone); oxazolidinones (including but not limited to linezolid); and others such as glycylcylcines, clofoctol, hexedine, methenamine, and nitroxoline.

The "adjunct administration" of a compound identified by the method of the invention and a second antibacterial compound means that the two are administered either as a mixture or sequentially. When administered sequentially, the compound may be administered before or after the second antibacterial compound, so long as the initially administered compound is still providing antibacterial activity. Any of the above described modes of administration can be used in combination to deliver the compound and the second antibacterial compound. When a compound identified by the method of the invention and a second antibacterial compound are administered adjunctively as a mixture, they are preferably given in the form of a pharmaceutical composition comprising both agents. Thus, in a further embodiment of the invention, it is provided a pharmaceutical composition comprising a compound of the invention and a second antibacterial compound together with a pharmaceutically acceptable carrier.

Target Infectious Agents

The antibiotic compounds identified by the methods of the infection can be used to treat infectious diseases in animals, including humans, companion animals (e.g., dogs and cats), livestock animals (*e.g.*, sheep, cattle, goats, pigs, and horses), laboratory animals (*e.g.*, mice, rats, and rabbits), and captive or wild animals.

Specifically, infectious diseases caused by bacteria including but not limited to, gram positive cocci, such as Staphylococci (*e.g., S. aureus*), Streptococci (*e.g., S. pneumoniae, S. pyrogens, S. faecalis, S. viridans*); gram positive bacilli, such as Bacillus (*e.g., B. anthracis*), Corynebacterium (*e.g., C. diphtheriae*), Listeria (*e.g., L. monocytogenes*); gram negative cocci, such as Neisseria (*e.g., N. gonorrhoeae, N. Meningitidis*); gram negative bacilli, such as Haemophilus (*e.g., H. influenzae*), Pasteurella (*e.g., P. multocida*), Proteus (*e.g., P. mirabilis*), Salmonella (*e.g., S. typhimurium*), Shigella species, Escherichia (*e.g., E. coli*), Klebsiella (e.g., *K. pneumoniae*), Serratia (*e.g., S. marcescens*), Yersinia (*e.g., Y. pestis*), Providencia species, Enterobacter species, Bacteroides (*e.g., fragilis*), Acinetobacter species, Campylobacter (*e.g., C. jejuni*), Pseudomonas (*e.g., P. aeruginosa*), Bordetella (*e.g., B. pertussis*), Brucella species, Fracisella (*e.g., F. tularensis*), Clostridia (*e.g., C. perfriugens*), Helicobacter (*e.g., H. pylori*), Vibrio (*e.g., V. cholerae*), Mycoplasma (*e.g., M. pneumoniae*), Legionella (*e.g., L. pneumophila*), Spirochetes (*e.g., Treponema, Leptospira* and *Borrelia*), Mycobacteria (*e.g., M. tuberculosis*), Nocardia (*e.g., N. asteroides*), Chlamydia (*e.g., C. trachomatis*), and Rickettsia species, can be treated by antibiotic drugs discovered by the methods of the invention.

The following examples serve to further typify the nature of the invention but should not be construed as a limitation in the scope thereof, which scope is defined solely by the appended claims.

5. EXAMPLES

In this section, examples of the methods described are provided for illustration only and not by way of limitation. The reactants and starting materials are either readily synthesized or purchased from commercial sources.

5.1 Example 1: Screen for identifying compounds that inhibit peptide deformylase.

An *E. coli* BL21 lysate (Promega, S30), containing endogenous peptide deformylase and aminopeptidase, was used to screen for compounds that inhibit PDF. The primary screen for peptide deformylase inhibition involved incubating the *E. coli* lysate with a formylated peptide substrate, formyl-methionine-alanine-serine-OH (fMAS), and the compound to be tested. Deformylase from the lysate deformylates the substrate peptide. After removal of the formyl group from the substrate, peptide aminopeptidase from the lysate cleaves the deformylated peptide into the three amino acids. A ninhydrin reagent, which reacts with the free amino groups of the methionine, alanine and serine produced by aminopeptidase, was then added to the reaction mixture and allowed for quantification of the reaction by measuring the absorbance at 570 nanometers. A decrease in the ninhydrin signal as compared to the control without peptide deformylase indicates that either deformylase or aminopeptidase was disrupted by the test compound.

Compounds were tested at 2-10 μg/ml in 4% DMSO. 3 μl of compound was added to the corresponding well in a 96 well plate. 3 μl of 0.625 mM 1,10 phenanthroline was added to the positive control wells. Using a 200 μl micropipettor, the contents of a vial of freshly thawed *E. coli* BL21 lysate was drawn up in the pipette tip gently so as to avoid bubbling. A full 200 μl of lysate may not be recovered from the vial so the micropipettor is dial is adjusted until the bubble at the tip is pushed out, this gives the actual volume of lysate. The lysate was gently ejected into a microfuge tube. Based on the actual volume, the lysate is diluted 1:3 in a tris-acetate buffer system (4.505 ml sterile H_2O, 50 μl 1M tris-acetate (pH 8.2), 140 μl 0.5 M magnesium acetate, 300 μl 1 M potassium acetate, 5 μl 1 M dithiothreitol). The buffer was not added directly into the lysate, but was added by using a micropipettor to allow the buffer to run down the side of the

microfuge tube into the lysate. The diluted lysate was mixed on a vortex mixer at a speed low enough to avoid introducing bubbles into the diluted lysate.

The lysate is stable for only 30-45 minutes at room temperature or on ice and must be used immediately after dilution. 4 μl of the diluted *E. coli* lysate was added to each test well including the control wells by placing the pipette tip on the side of the well to allow the lysate to run around the perimeter of the well. When the lysate was ejected from the pipette it was only pushed to the first stop to avoid introducing bubbles into the reaction mixture. The lysate was not mixed at this stage. 8 μl of the fMAS substrate was then added to each well except for the background wells which received 8 μl of dH_2O. The 8 μl of fMAS was added by placing the pipette tip at the bottom of the well. The fMAS was slowly ejected until the first stop and then drawn up and down 3-4 times very slowly to mix the entire reaction. All of the liquid from the pipette tip was not ejected at the end of the mixing. At this point the plate of reaction mixtures had few or no bubbles. The test plates were covered and then incubated for exactly 1 hour at 30 °C in a water bath. The incubation was then followed by the addition of 120 μl of ninhydrin reagent (0.86 g ninhydrin was dissolved in 25 mls ethanol followed by addition of 25 mls DEPC H_2O). The plates were covered with plastic adhesive covering, vortexed on low for 10 seconds and incubated for 20 minutes at 45 °C in a water bath. The optical density at 570 nanometers was determined in the spectrophotometer (Spectramax 250). Background wells contained 3 μl 4% DMSO, 4 μl lysate and 8 μl water. Phenanthroline inhibitor wells contained 3 μl 0.625 mM phenantroline, 4 μl lysate and 8 μl fMAS. No compound wells contained 3 μl 4% DMSO, 4 μl lysate and 8 μl 8.44 mM fMAS. The minimum inhibitory concentration was then determined.

5.2 Example 2: Determination of selectivity profiles for peptide deformylase inhibitors with peptide deformylase containing the native iron catalytic metal center.

Compounds identified in the initial screen were used to determine their binding affinity to PDF in a novel assay that utilizes the enzymes native iron metal center and the binding affinity to several other metalloproteases including thermolysin, collagenase, carboxypeptidase and angiotensin converting enzyme. The selectivity profile is then determined by comparing the binding affinity of the inhibitor to peptide deformylase and other metalloproteases which include thermolysin, collagenase, carboxypeptidase, and angiotensin converting enzyme. Compounds that bind to PDF with a greater than or equal to 10 fold affinity relative to the other metalloproteases are identified as antimicrobials that selectively bind PDF.

The percent Fe-PDF inhibition assays were set up in 96-well polypropylene plates with 12 columns. Column 1 was reserved for the negative control (20% DMSO), columns 2-11 were for test compounds and column 12 was for the positive control (actinonin). The following reagents were prepared the day of use; 20% DMSO, 100 mM TCEP-HCl (0.129 g of TCEP-HCl bring to 4.5 ml total volume with 50 mM potassium phosphate pH 7), dilution buffer for Fe-PDF (0.5 ml 100 mM NaCl, 0.5 ml 10 mg/ml BSA, 0.5 ml 100 mM TCEP-HCl and 3.5 ml 0f 50 mM potassium phosphate pH 7) The reaction mixture was prepared by adding the following components, in the order indicated, into a 15 ml polypropylene tube; 1404 μl CHELEX (Sigma) treated water, 972 μl 100 mM NaCl,972 10 mg/ml BSA, 972 μl 100 mM TCEP-HCl (Peirce), 1080 μl 100 mM fMAS, 2160 μl 100 mM NAD, 1620 U/ml formate dehydrogenase (FDH, Roche). The reaction mixture was agitated and using a 12 channel micropipette, 85 μl of the reaction mixture was added to each well. The concentration of the test compounds stock solution was 30 mM. The test compounds were diluted in a working 96 well plate by adding 6 μl of the stock test compound solution

to columns 2-11 of the working test plate followed by the addition 24 μl of CHELEX treated water to each well containing test compounds. 50 μl of 20% DMSO was added to each well of in column 1 for the negative control. A 1/5 serial dilution of the 13 μM actinonin stock solution was prepared through the addition of 40 μl of CHELEX treated water and 10 μl 13 μM actinonin. 25 μl of the 1/5 dilution of actinonin was added to the next well along with 25 μl of 20% DMSO to give a 1/10 dilution. The 1/10 dilution was used to make a 1/20 dilution and this processes was iterated until a 1/640 dilution was prepared in the last well for the actinonin positive controls.

The 96 well reaction plate was prepared by using a 12 channel micropipetter to transfer 5 of the contents of each well from columns 1-12 of the working test plate to the reaction plate. 85 μl of the reaction mixture was then added to each well on the reaction plate containing the negative control, the 6 mM test compound and the positive control. Endpoint data was recorded in the Spectromax 250 spectrophotometer by monitoring at 340 nanometers. The serial dilutions of Fe-PDF are prepared (as described in section 4.1, see Rajagopalan et al., (1997) J. Am. Chem. Soc. 119:12418-12419; Rajagopalan et al., (1997) Biochemistry 36:13910-13918) usually during the endpoint reading, just prior to use in the reactions. The Fe-PDF was diluted 1/20,000 with the dilution buffer. The buffer is necessary for stabilizing the diluted enzyme. 10 μl of the 1/20,000 fold diluted Fe-PDF was added to each well containing the reaction mixture along with the negative control, the test compounds and the positive control. The reaction plate was then mixed on a vortex mixer at low speed for 5 seconds. The reactions were then monitored in the Spectromax 250 spectrophotometer. Quantification of results from these experiments allowed for the determination of percent inhibition of Fe-PDF at 300 μM test compound.

The assays to determine the binding affinity of the test compounds were based on the results obtained from the percent

Fe-PDF inhibition studies. The initial concentrations of the test compounds in the binding affinity assays were determined as follows:

(1) Initial concentrations for test compounds exhibiting between 50-88 percent Fe-PDF inhibition were 300 μM;
(2) Initial concentrations for test compounds exhibiting between 88-92 percent Fe-PDF inhibition were 150 μM;
(3) Initial concentrations for test compounds exhibiting between 92-96 percent Fe-PDF inhibition were 60 μM;
(4) Initial concentrations for test compounds exhibiting between 96-100 percent Fe-PDF inhibition were 30 μM;
(5) Initial concentrations for test compounds exhibiting between 100 percent Fe-PDF inhibition were 15 μM;
(6) Highly active compounds required testing at initial concentrations lower than 15 μM.

Compounds were initially prepared in a 96 well working plate with 12 columns. Column 1 was for the negative control (20% DMSO), columns 2-11 were for the serial dilutions of the test compounds and column 12 was for the serial dilutions of the positive control, actinonin. Row A, columns 2-11, received 40 μL of CHELEX treated water and 10 μL of test compound. 25 μL of 20% DMSO was added to rows B-H, columns 1-12 of the working plate. 25 μL of the 1/5 dilution of the test compound in row A was then added to Row B, giving a 1/10 dilution. This process was interated until the last row which was a 1/640 fold dilution. Column 1 received 20% DMSO and column 12, the positive control actinonin, was diluted in tha same manner as the test compounds providing 1/5-1/640 serial dilutions of 13 μM actinonin. Using a 12 channel micropipetter 5 μL of columns 1-12, row H of the working plate was added to row H of the reaction plate. 85 μL of reaction mixture, as described above, was added to each well. Next the plate was incubated in the spectrophotometer for 3-5 minutes while the serial dilutions of the Fe-PDF were made as described above The reactions were initiated by addition of 10 μL of 1/20,000 fold dilute Fe-PDF. After addition of the

Fe-PDF the plate was immediately inserted into the Spectramax 250 spectrophotometer. Analysis of the data from the spectrophotometer readings provided the binding affinities for the test compounds.

5.3 Example 3: Metalloprotease Selectivity Assay and Profiles.

Carboxypeptidase A Selectivity Assay

Test compounds at 30 mM initial concentration in DMSO were diluted 10-fold in dH_2O to 3 mM. The DMSO concentration is 10%. Serial dilutions in 2-fold increments were made to give from 3 mM to 23.4 μM. Reactions were run in 96-well plates with one column for the positive control, another column for the negative control and the rest for test compounds. 20 μl of appropriately diluted drug was added to the test compound wells. The negative control wells received 20 μl of 10% DMSO. One well that received 20 μl of 10% DMSO also receives 20 μl of 50 mM Tris + 450mM NaCl buffer (pH 7.5). This was the blank well (no enzyme). The wells in one column of the plate received 20 μl of the Carboxypeptidase A control inhibitor in the following concentrations: 3.0 mM, 1.5 mM, 0.75 mM, 0.375 mM, 0.188 mM, 0.094 mM, 0.047 mM, 0.023 mM. 500 μl of stock enzyme (1 U/ml) was diluted with 1.5 ml of 50 mM Tris + 450mM NaCl buffer (pH 7.5) to obtain a final concentration of 0.25 U/ml Carboxypeptidase A. 20 μl of enzyme was transferred to wells containing compound. The plates were gently mixed and allowed to incubate for 5 min at room temperature. 160 μl of the substrate (0.5 mM), FAPP (furanacroyl peptide, FA-Phe-Phe-OH), was added to all wells on the test plate. Immediately after the substrate was added the plates were placed in the spectrophotometer and read at 330 nm. Once plate was read it was incubated at room temperature for 60 min. After the plate incubated for 60 min another reading was obtained in the spectrophotometer at 330 nm.

Collagenase Selectivity Assay

Test compounds were diluted as described in the Carboxypeptidase A assay and set up in a 96 well plate. 20 μl of appropriately diluted drug was added to the test compound wells. Positive control wells had the collagenase control inhibitor at the following concentrations: 3 mM, 100 μM, 30 μM, 10 μM, 3 μM, 1 μM, 0.3 μM, 0.1 μM. 20 μl of stock enzyme (15 U/μl) was diluted into 1.98 ml of 50 mM tricine buffer (50 mM tricine pH 7.5 + 400 mM NaCl and 10 mM $CaCl_2$) to obtain a final concentration of 150 U/ml Collagenase. 20 μl of the diluted enzyme was transferred to wells containing compound. The plates were gently mixed and allowed to incubate for 5 min at room temperature. 160 μl of the substrate (0.5 mM in 1 M tricine pH 7.5), FALGPA (furanacroyl peptide, FA-Leu-Gly-Pro-Ala-OH) was added to all the wells on the test plate. Immediately after the substrate was added the plates were placed in the spectrophotometer and read at 330 nm. Once the plate was read it was removed and was incubated at room temperature for 15 min. After plate incubated for 15 min another reading was obtained in the spectrophotometer at 330 nm.

Thermolysin Selectivity Assay

Test compounds were diluted as described in the Carboxypeptidase A assay and set up in a 96 well plate. 20 μl of appropriately diluted drug was added to the test compound wells. Positive control wells had the thermolysin control inhibitor phosphoramidon at the following concentrations: 1 mM, 300 μM, 100 μM, 30 μM, 10 μM, 3.0 μM, 1.0 μM, 0.3 μM. 25 μl of 1 mg/ml thermolysin was diluted into 9.975 ml of borate buffer (250 mM)for a concentration of 2.5 μg/ml thermolysin. 45 μl of the diluted enzyme was transferred to wells containing compound. The plates were gently mixed and allowed to incubate for 5 min at room temperature. 10 mg/ml succinylcasein was prepared by dissolving 50 mg of succinylcasein in 5 ml borate buffer. 45 μl of succinylcasein was added to all wells on the test plate. The plates were

covered and incubated at 37°C for 45 min. 50 µl of the stopping buffer (100mM sodium borate, 100 mM NaOH and 5% SDS)was added after the 45 minute incubation. Do not mix because solution will cause bubbles. The reactions were allowed to stop at room temperature for 5 min. 50 µl of 0.03% TNBSA working solution (2,4,6-trinitrobenzene sulfonic acid in methanol) was added to all wells and was not mixed. The plates were covered and incubated at 37°C for 30 min. Once the TNBSA incubated for 30 min the plates were placed in the spectrophotometer and read at 430 nm.

Angiotensin Converting Enzyme Selectivity Assay (ACE)

Test compounds were diluted as described in the Carboxypeptidase A assay and set up in a 96 well plate. 20 µl of appropriately diluted drug was added to the test compound wells. 10 µL of the positive control Captopril was added to positive control wells to give the following concentrations: 1.01 µM, 0.32 µM, 0.1 µM, 0.032 µM, 0.01 µM, 0.0032 µM, 0.001 µM, 0.0003 µM. 1.0 ml of Tris buffer (1 M, pH 7.5) was added to one vial of ACE (0.1 U) giving an enzyme concentration of 0.1U/ml. The vial was carefully mixed and allowed to sit on ice for 15 min followed by mixing again. 10 µl of the enzyme was transferred to wells containing compound. The reactions were allowed to stop at room temperature for 5 min. 80 µl of 0.25 mM substrate Abz-Gly-Phe(NO_2) (in 50 mM tris pH 7.5 and 100 mM NaCl) was added to all the wells on the test plate. Once the substrate was added the plates were immediately placed the plate in the Spectrophotometer and read at Ex:360 nm, Em:410 nm. After the plate was read remove it was allowed to incubate at room temperature for 60 min. Once the plate had incubated for 60 min it was analyzed in the spectrophotometer again.

Table 1

Compound	Inhibition at 300 μM Test Compound[2]					
	Selectivity Index[1]	Fe PDF	THERM	CPEP	COLL	ACE
1	506	100	0.08	0.96	0.74	1.01
2	1260	100	0.09	1.01	0.07	0.95
3	190	100	-0.02	1.04	0.75	0.64
4	3702	100	0.07	1.11	0.12	0.88
5	> 30	100	0.95	1.03	0.85	0.32
6	> 27	98.3	0.98	0.97	0.84	0.15
7	181	100	0.1	1.03	0.87	0.56
8	81	101	0.02	0.95	0.08	1.02
9	1426	100	0.03	1.06	0.03	0.96
10	1932	100	0.06	0.96	0.65	1.1
11	837	108	0.1	0.98	0.75	1.18
12	560	100	0.38	1.01	0.76	0.91
13	3539	100	0.01	0.93	0.80	1.45
14	841	100	0	0.99	0.65	0.95
15	3727	100	0.12	0.98	0.50	1.09
16	1048	100	0.06	1.08	0.22	0.36
17	392	100	0.02	1.06	0.45	1
18	128	102	0.01	0.95	0.80	1.02

[1] The selectivity of the inhibitors was calculated as the ratio of the IC_{50} for the compound versus thermolysin (THERM) divided by the IC_{50} for the compound versus peptide deformylase (Fe-PDF). The metalloproteases carboxypeptidase (CPEP), collagenase (COLL) and angiotensin converting enzyme (ACE) were also used in these studies but the IC_{50} for

thermolysin (THERM) was generally the smallest for these enzymes.

[2] For Fe-PDF inhibition is given as percent inhibition and for THERM, CPEP, COLL and ACE the inhibition values are the inhibition relative to controls with no inhibitor (enzymatic reaction with test compound divided by reaction without test compound).

Table 2

Compounds with Antibacterial Activity

Compound	Fe-PDF IC50 (μM)	MIC SA (μg/ml)[1]	MIC EC (μg/ml)[1]
1	0.010	25	100
2	0.006	50	>100
3	0.0341	25	>100
4	0.0074	25	>100
5	13.3	50	>100
6	10.77	50	>100
7	0.102	25	>100
8	5.93	25	100
9	0.0064	25	>100
10	0.054	12.5	50
11	0.047	25	25
12	0.010	50	>100
13	0.011	12.5	50
14	0.044	12.5	50
15	0.0060	50	>100
16	0.0150	25	>100
17	0.011	25	>100
18	0.03	6.25	25

[1] SA represents *Staphylococcus aureus* and EC is *Escherichia coli*.

5.4 Example 4: Synthesis of Compound 1.

General

1H and 13C NMR spectra were recorded on a 300 MHz FT-NMR. Preparative HPLC purification was performed on a Gilson HPLC System with 215 autosampler/faction collector using a Hewlett-Packard 50 x 21.2 mm XDB C18 reverse-phase column running acetonitrile/water gradients at 25 ml/min. Analytical HPLC/MS (electrospray) was performed on a Hewlett-Packard 1100 HPLC with MSD using a MetaChem MetaSil AQ C18 column running acetonitrile/water gradients at 2 ml/min. All anhydrous solvents were purchased from Aldrich chemical company in SureSeal containers. Most reagents were purchase from Aldrich Chemical Company. Protected amino acids were obtained from NovaBiochem.

Abbreviations: Boc, *tert*-butoxycarbonyl; DCM, dichloromethane; DIEA, N,N-diisopropylethylamine; EDC, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; TFA, trifluoroacetic acid.

Array synthesis was conducted in 15 x 75 mm glass round bottom screw-cap vials contained in a custom 4 x 6 array aluminum synthesis block, sealed with a Teflon-lined rubber membrane. Reagents were added and aqueous extractions performed with single or multichannel pipettors. Filtrations were performed using Whatman/Polyfiltronics 24 well, 10 ml filtration blocks on a Whatman/Polyfiltronics UniVac filtration manifold modified to collect eluent in the custom synthesis block. Evaporation of volatile materials from the array was performed with a Labconco Vortex-Evaporator, unless indicated otherwise.

Compounds 1-4, 7, 9, 10, 12, and 15-17 were prepared in a parallel array format with the procedures as indicated. Individual examples may also be obtained by individual synthetic procedures using adaptations of the experimental details shown

here, which would be readily accomplished by one skilled in the art.

Array synthesis procedure

Six Boc-protected amino acids (Val, Nva, Phe, Met, Ser(OBn), Tyr(OBn)) were each dissolved in DCM to a concentration of 1 M, and 4 aliquots (0.48 ml, 0.48 mmol) of each were added to each column of the array, one per vial. Three of the starting amine reagents (pyrrolidine, piperidine, and benzylamine) were each dissolved in DCM at 2.0 M. Dimethylamine was used as provided, in a 2 M THF solution (Aldrich). Six aliquots (0.30 ml, 0.6 mmol) of each of the 4 amine solutions were added to each row of the array, one per vial. A 0.25 M solution of EDC in DCM was prepared and an aliquot (2.40 ml, 0.6 mmol) was added to each vial. The vials were sealed in the block and shaken at 200 rpm on a rotary shaker for 18 h. To each vial was added 1.5 ml 10% $NaHSO_4$ (aq), the array was sealed with the membrane, shaken, and the aqueous layers were withdrawn. The wash procedure was repeated with 10% $NaHSO_4$, $NaHCO_3$, and brine. The solutions were filtered through approx. 0.5 g anhydrous Na_2SO_4 each and evaporated to dryness. The resulting Boc-protected amides (oils and solids) were placed in a vacuum chamber under high vacuum overnight, weighed, and analyzed by HPLC/MS.

Deprotection of the Boc-protected amino amides was accomplished by dissolving each product in 2 ml of 4 M HCl in dioxane, shaken for 45 min, and evaporated. The vials were then placed under high vacuum overnight, weighed, and the amine hydrochlorides were analyzed by HPLC/MS.

To each of the amine hydrochlorides was added a solution of (R) *tert*-butyl (2-pentyl)succinate mono acid in DCM (2.0 M, 0.20 ml, 0.40 mmol), EDC in DCM (0.25 M, 2.4 ml, 0.60 mmol), and DIEA (209 ml, 1.2 mmol). The mixtures were shaken for 18 h, washed twice with 10% $NaHSO_4$ (aq), once each with $NaHCO_3$, and brine, filtered through Na_2SO_4 and evaporated. The resulting *tert*-butyl ester substituted amides were then weighed and analyzed by HPLC/MS.

The *tert*-butyl esters were converted to the carboxylic acids by dissolving each in 2 ml of 4 M HCl in dioxane, shaking for 45 min, and evaporating. The vials were then placed under high vacuum overnight, weighed, and the acids were analyzed by HPLC/MS.

To convert the acids to the corresponding methyl esters each was dissolved in 0.3 ml of methanol and 0.7 ml of benzene, and trimethylsilyldiazomethane in hexanes (2.0 M, 0.60 ml, 1.2 mmol) was added slowly. The array was shaken for 2 h, the solutions were evaporated, and the residues placed under high vacuum overnight. The resulting methyl esters were weighed and analyzed by HPLC/MS.

A methanolic solution of NH_2OH HCl (2 M, 30 ml) was cooled to 0 °C. A methanolic solution of KOH (3 M, 30 ml) was added dropwise over 30 min, the mixture was stirred for 30 min and filtered. An aliquot (2 ml, 2.0 mmol NH_2OH) of this solution was added to each of the reaction vials containing the methyl ester product of the previous reaction. The array was shaken for 30 min. Each solution was partially neutralized with 0.10 ml of conc. HCl, then quickly buffered with 0.30 ml of 1 M triethylammonium bicarbonate (pH 7). The solutions were then filtered and directly purified by preparative HPLC (2 injections per compound). The product-containing fractions were collected, evaporated in a Savant evaporating centrifuge, and re-analyzed by HPLC/MS.

An exemplary specific synthesis of compound 1 is illustrated in the following schematic and described below for compound.

A DCM solution of Boc-Phe (1 M, 0.48 mL, 0.48 mmol) was added to a glass vial. A solution of 2 M dimethylamine in THF (0.30 mL, 0.6 mmol) was added, followed by a 0.25 M solution of EDC in DCM (2.40 mL, 0.6 mmol). The vial was sealed with a Teflon-lined rubber membrane and shaken at 200 rpm on a rotary shaker for 18 h. The solution was washed twice with 1.5 mL 10% $NaHSO_4$ (aq), and once with $NaHCO_3$ (sat) and brine. The solution dried over anhydrous Na_2SO_4, filtered and evaporated. The Boc-protected amides (oils and solids)

were then placed in a vacuum chamber under high vacuum overnight, weighed, and analyzed by HPLC/MS.

The Boc-protected amino amide was dissolved in 4 M HCl in dioxane, shaken for 45 min, and evaporated. The vial were then placed under high vacuum overnight, weighed, and analyzed by HPLC/MS.

To the amine hydrochloride salt of the previous reaction was added a solution of (R) *tert*-butyl (2-pentyl)succinate mono acid in DCM (2.0 M, 0.20 mL, 0.40 mmol), EDC in DCM (0.25 M, 2.4 mL, 0.60 mmol), and DIEA (209 mL, 1.2 mmol). The mixture was shaken for 18 h, washed twice with 10% $NaHSO_4$ (aq), once with $NaHCO_3$ (sat) and brine, filtered through Na_2SO_4 and evaporated. The t-butyl ester was then weighed and analyzed by HPLC/MS.

The t-butyl ester was dissolved in 4 M HCl in dioxane, shaken for 45 min, and evaporated. The vial was placed under high vacuum overnight, weighed, and analyzed by HPLC/MS.

The acid product was dissolved in 1 mL 30% methanol in benzene, and trimethylsilyldiazomethane in hexanes (2.0 M, 0.60 mL, 1.2 mmol) was added slowly The solution was shaken for 2 h, evaporated and placed under high vacuum overnight. The methyl ester product was weighed and analyzed by HPLC/MS.

A methanolic solution of NH_2OH HCl (2 M, 30 mL) was cooled to 0 °C. A methanolic solution of KOH (3 M, 30 mL) was added dropwise over 30 min, and the mixture was stirred for 30 min and filtered. An aliquot (2 mL, 2.0 mmol) of this solution was added to the reaction vial containing the methyl ester product of the previous reaction, and shaken for 30 min. The solution was neutralized with 100 mL conc. HCl, then quickly buffered with 300 mL 1 M triethylammonium bicarbonate (pH 7). The solution was then filtered and purified by preparative HPLC using a Hewlett-Packard XDB C18 reverse-phase column. Fractions were analyzed by HPLC/MS and the product was collected by evaporation in a Savant evaporating centrifuge and re-analyzed by HPLC/MS.

Calculated molecular weight 377.45, Found 345.1, 378.2, 400.1 (M-NHOH, M+H, M+Na)(M-NHOH is the mass of the molecular ion minus hydroxamic acid, NHOH, M+H is the molecular ion plus a proton and M+Na is the molecular ion plus sodium).

Synthesis of Compound 2: Calculated molecular weight 407.48, Found 375.2, 408.3, 430.2 (M-NHOH, M+H, M+Na).

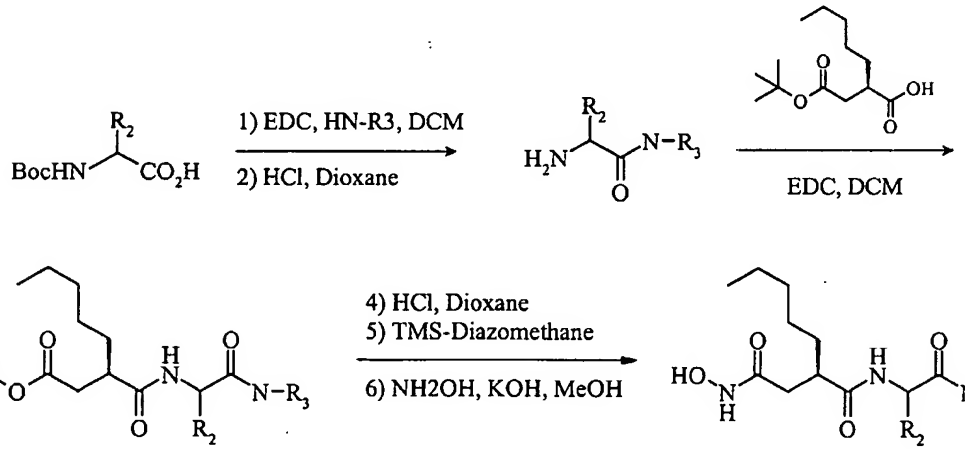
Synthesis of Compound 3: Calculated molecular weight 391.54, Found 359.2, 392.3, 414.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 4: Calculated molecular weight 391.54, Found 359.2, 392.3, 414.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 7: Calculated molecular weight 447.57, Found 415.3, 448.2, 470.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 9: Calculated molecular weight 433.54, Found 401.2, 434.2, 456.1 (M-NHOH, M+H, M+Na).

Synthesis of Compound 10: Calculated molecular weight



355.50, Found 323.2, 356.3, 378.13 (M-NHOH, M+H, M+Na).

Synthesis of Compound 12: Calculated molecular weight 483.57, Found 451.2, 484.2, 506.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 15: Calculated molecular weight 387.50, Found 355.1, 388.1, 410.1 (M-NHOH, M+H, M+Na).

Synthesis of Compound 16: Calculated molecular weight 509.63, Found 345.1, 378.2, 400.1 (M-NHOH, M+H, M+Na).

Synthesis of Compound 17: Calculated molecular weight 403.51, Found 371.2, 404.2, 426.2 (M-NHOH, M+H, M+Na).

5.5 Example 5: Synthesis of compounds 8, 11, 13-14, 18

Compounds 8, 11, 13-14, 18 were synthesized in parallel according to the following procedure described for compound 11. See schematic after description.

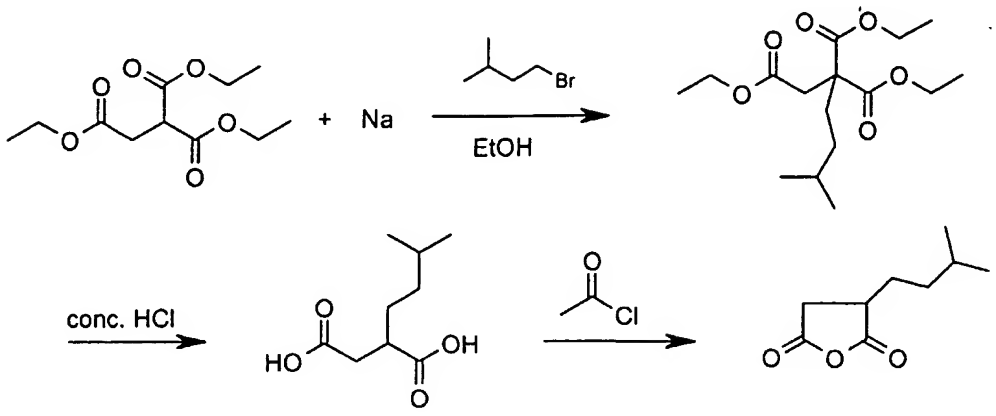
Synthesis of 3-carboxy-6-methyl heptanoic acid. 5.6 g (17.7mmol) Ethyl 3,3-di(ethylcarboxy)-6-methylheptanoate was added to 55 mL con. HCl and heated to reflux for 2 days. White solid precipitate was noted. The reaction mixture was poured into ice and extracted with ether. Combined extracts were dried ($MgSO_4$) and concentrated to form a yellow oil. The oil crystallized upon standing to give 2.5 g (92%) of 3-carboxy-6-methyl heptanoic acid, a colorless crystalline solid.

Synthesis of isopentyl succinic anhydride. 2.8g (14.9 mmol) of 3-carboxy-6-methyl heptanoic acid was added to 5.2 mL acetyl chloride and heated to reflux for 3 hr. Volatile material was removed in vacuo to give 2.5 g crude isopentyl succinic anhydride which was used without further purification.

Synthesis of N-Boc-Valine homopiperidine amide. 3.3 g (33.2 mmol) of homopiperidine was added to a solution of 6.0 g (27.7 mmol) N-Boc-Valine and 6.3g (33.2 mmol) EDC in DCM (60 mL). The mixture was stirred for 16 hours. The reaction mixture was diluted with DCM, washed with water, brine, dried ($MgSO_4$) and concentrated. Residue was purified by flash chromatography using 60% EtOAc in hexanes to give 3.4 g N-Boc-Valine homopiperidine amide as a colorless oil.

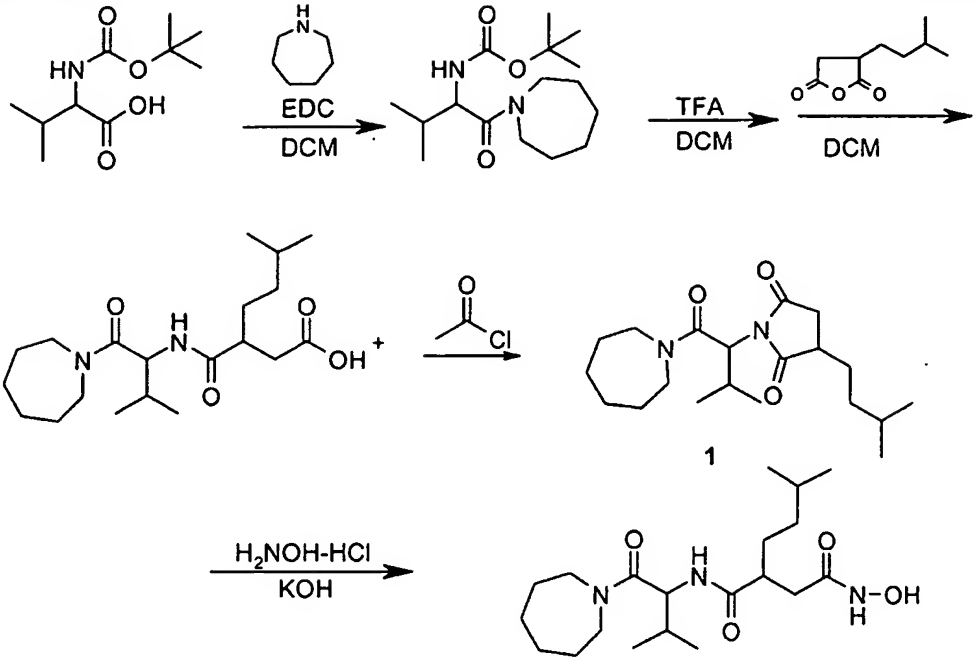
Coupling of isopentyl succinic anhydride and isopentyl succinic anhydride. 3 mL TFA was added to a solution of 0.650 g (2.2 mmol) N-Boc-Valine homopiperidine amide in DCM (12 mL). The ice bath was removed and solution stirred for 4.5 h. Solvent was removed in vacuo and replaced with DCM (20 mL). 0.341 g (2.6 mmol) DIEA was added to the solution followed by addition of 0.442 g (2.6 mmol) isopentyl succinic anhydride. The solution was stirred for 3 days. The mixture was diluted with DCM, washed with brine, dried

($MgSO_4$) and concentrated. Residue was purified by flash chromatography using EtOAc. 0.740 g (91%) of product was obtained as a colorless oil.



Synthesis of Compound 11. 0.740 g (2.0 mmol) coupling product from the previous reaction was added to 0.71 mL acetyl chloride and heated to reflux for 3 h. EtOAc was added and washed with sat $NaHCO_3$ (x2), brine, dried $MgSO_4$) and concentrated. A cold solution of 0.264 g (3.8 mmol) hydroxamic acid hydrochloride salt in MeOH was treated with a solution of 0.319 g (5.7 mmol) KOH and let stand for 15 minutes at 0 °C. The resulting solution was filtered, added to the residue from the previous step and stirred at rt for 2 days. The pH of the solution was neutralized to pH ~ 6 by addition of Dowex 50W-X8 resin. The solution was filtered and concentrated. The residue was purified by flash chromatography using 80% EtOAc in hexanes.
Calculated molecular weight 383.25, Found 351.2, 384.2, 406.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 8: Calculated molecular weight 375.25, Found 343.2, 376.3, 398.25 (M-NHOH, M+H, M+Na).



Synthesis of Compound 13: Calculated molecular weight 381.25, Found 349.2, 382.3, 404.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 14: Calculated molecular weight 339.25, Found 307.2, 340.3, 362.2 (M-NHOH, M+H, M+Na).

Synthesis of Compound 18: Calculated molecular weight 341.25, Found 309.2, 342.3, 364.2 (M-NHOH, M+H, M+Na).

5.6 Example 6: Synthesis of Compound 5.

Ethyl 2-(3-Nitrobenzenesulfonylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate A mixture of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (200mg, 0.89mmol), 3-nitrobenzenesulfonyl chloride (295mg, 1.33mmol), and pyridine (4.4mL) was heated under reflux for 1h. The reaction mixture was concentrated in vacuo. $CHCl_3$ was added to the residue, then washed with 1N HCl, and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by preparative TLC (silica gel, EtOAc/n-hexane, 1:4), and recrystalization (CH_2Cl_2/n-hexane) to give the title compound (66mg). ^1H NMR (300MHz, DMSO-d_6) d: 1.2 (t, 3H, J=7.0Hz), 1.65-1.8 (m, 4H), 2.5-2.7 (m, 4H), 4.09 (q, 2H, J=7.0Hz), 7.9-8.0 (m, 1H), 8.17-8.23 (m, 1H), 8.5-8.6 (m, 2H), 10.87 (br s, 1H) . MS: m/z: 409 ([M-H]$^-$).

5.7 Example 7: Synthesis of Compound 6.

Ethyl 2-(4-Nitrobenzenesulfonylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate A mixture of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (3g, 13.3mmol), 4-nitrobenzenesulfonyl chloride (4.4g, 19.9mmol), and pyridine (66mL) was heated under reflux for 1.5h. The reaction mixture was concentrated in vacuo. $CHCl_3$ was added to the residue, then washed with 1N HCl, and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by chlomatography (silica gel, EtOAc/n-hexane 20:1 to 3:1) to give the title compound (1.78g). ^{1}H NMR (300MHz, DMSO-d_6) d: 1.22 (t, 3H, J=7.0Hz), 1.65-1.85 (m, 4H), 2.55-2.7 (m, 4H), 4.12 (q, 2H, J=7.0Hz), 8.02-8.1 (m, 2H), 8.43-8.51 (m, 2H), 10.88 (br s, 1H) . MS m/z: 409 ([M-H]$^-$).

We claim:

1. A method of screening for test compounds that selectively inhibit peptide deformylase containing the native iron catalytic metal center which comprises:

a) placing the test compound in an assay which detects inhibition of the peptide deformylase;

b) incubating the test compound in the assay for a time sufficient to allow the test compound to inhibit the deformylase and;

c) measuring the level of the deformylase activity so that the level of deformylase activity indicates whether the test compound inhibits the peptide deformylase.

2. The method of claim 1 which further comprises determining the binding affinity of the test compounds shown to inhibit the deformylase and detecting those compounds which have a greater affinity to the deformylase than to one or more of the following metalloproteases: thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

3. The method of claim 2 wherein compounds are detected which have a greater than or equal to 10-fold affinity for the deformylase than for one or more of the following metalloproteases: thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

4. The method of claim 3 wherein compounds are detected which have a greater than or equal to 100-fold affinity for the deformylase than for one or more of the following metalloproteases: thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

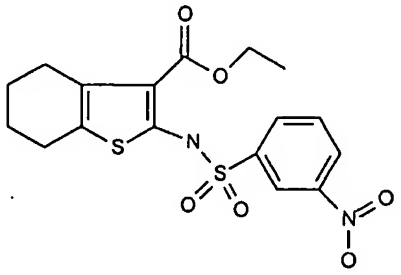
5. A method of treating bacterial infections in a subject in need thereof with a therapeutically effective amount of a compound which selectively inhibits native peptide deformylase identified from the methods of claims 1,2,3 or 4.

6. The method of claim 5 wherein the compound binds the native deformylase with greater affinity than said compound binds to the one or more of following metalloproteases: thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

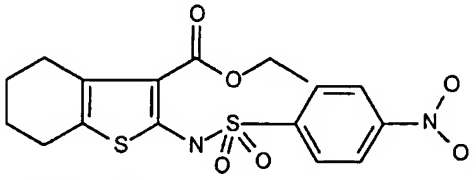
7. The method of claim 6 wherein the compound binds to the native peptide deformylase with a greater than or equal to 10-fold affinity than said compound binds to one or more of the following metalloproteases: thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

8. The method of claim 7 wherein the compound binds to the native peptide deformylase with a greater than or equal to 100-fold affinity than said compound binds to one or more of the following metalloproteases: thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme.

9. A method of treating a bacterial infection in a subject in need thereof with a compound selected from the group consisting of:



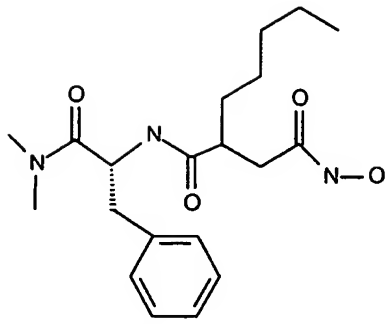
Compound 5



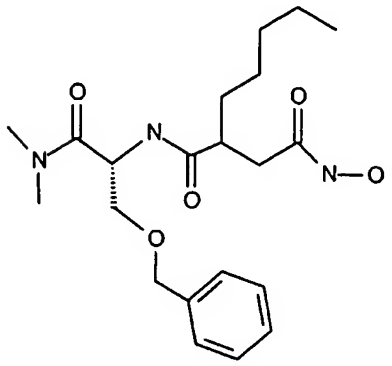
Compound 6

or a pharmaceutically acceptable salt thereof.

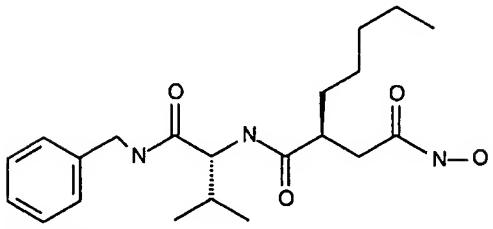
10. A method of treating a bacterial infection in a subject in need thereof with a compound selected from the group consisting of:



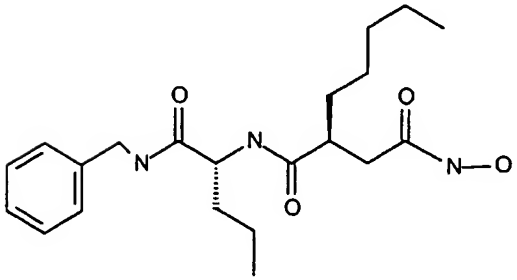
Compound 1



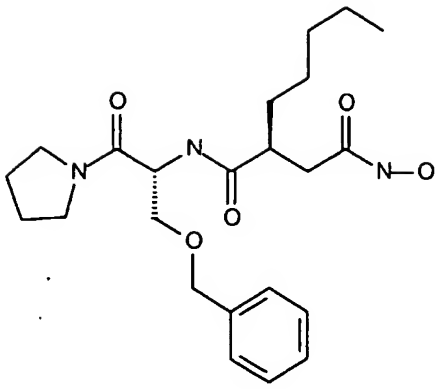
Compound 2



Compound 3

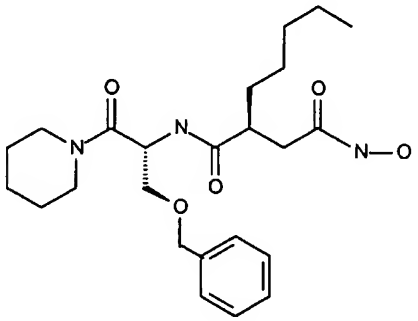


Compound 4

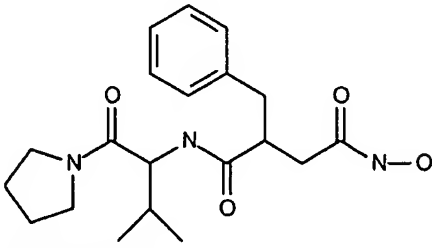


Compound 9

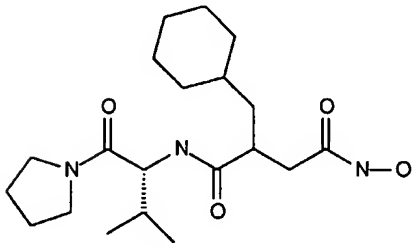
11. A method of treating a bacterial infection in a subject in need thereof with a compound selected from the group consisting of:



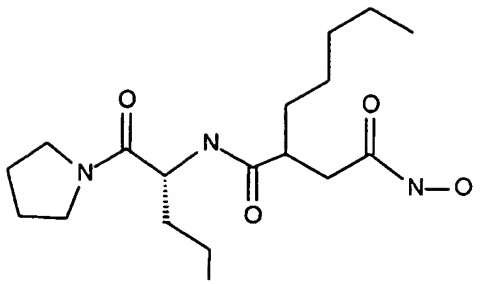
Compound 7



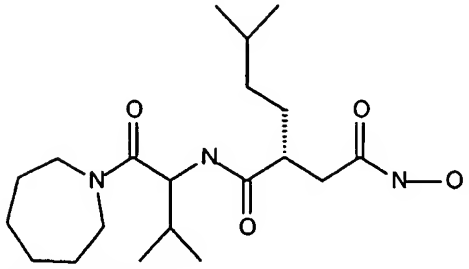
Compound 8



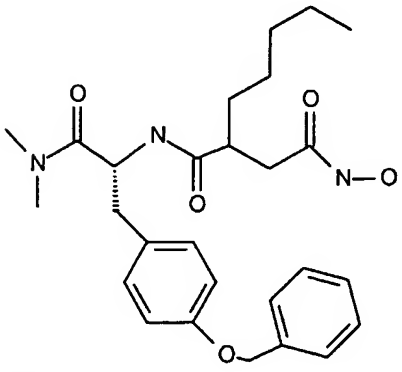
Compound 13



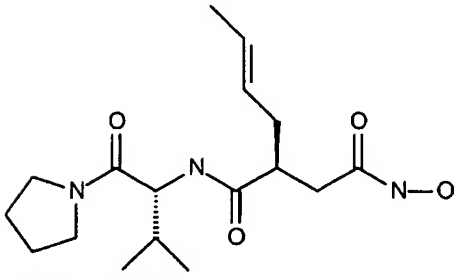
Compound 10



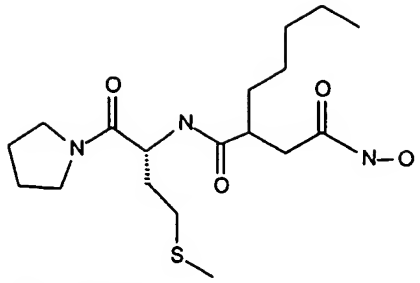
Compound 11



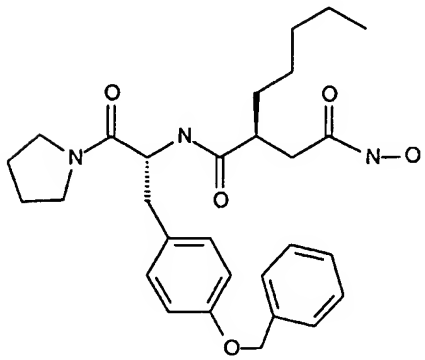
Compound 12



Compound 14



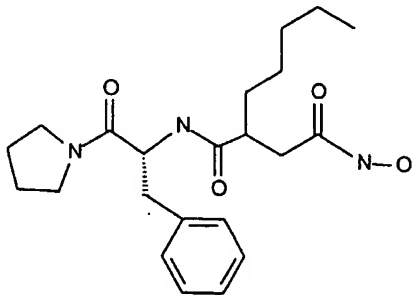
Compound 15



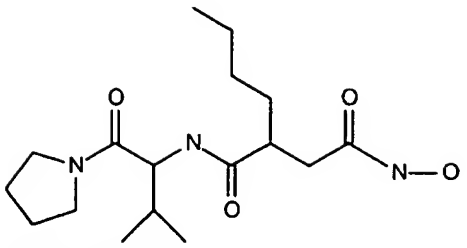
Compound 16

or a pharmaceutically acceptable salt thereof.

12. A method of treating a bacterial infection in subject in need thereof with a compound which binds to peptide deformylase with its native catalytic iron center with a greater than or equal to 100-fold affinity than it binds to any of the following metalloproteases thermolysin, carboxypeptidase, collagenase and angiotensin converting enzyme; and is further selected from the group consisting of:



Compound 17



Compound 18

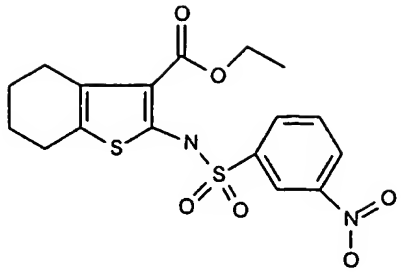
or a pharmaceutically acceptable salt thereof.

13. The method of claims 5, 6, 7, 8, 9, 10, 11 or 12 further comprising adjunctively administering to the subject a second antibacterial compound.

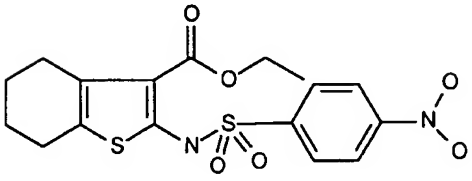
14. The method of claim 13 wherein the second antibacterial compound is a member of an antibiotic group selected from the group consisting of aminoglycosides, amphenicols, ansamycins, β-lactams, cephalosporins, cephamycins, monobactams, oxacephems, penicillins, lincosamides, macrolides, polypeptide antibiotics, tetracyclines, 2,4-diaminopyrimidines, nitrofurans, quinolones, streptogramins, sulfonamides, sulfones, oxazolidinones and glycylcyclines.

15. A pharmaceutical composition comprising a compound which inhibits peptide deformylase with the native iron catalytic metal center and a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising a compound which inhibits peptide deformylase with the native iron catalytic metal center that is selected from the group consisting of:

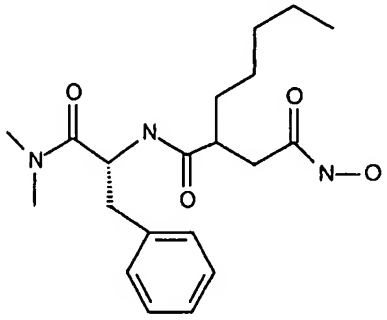


Compound 5

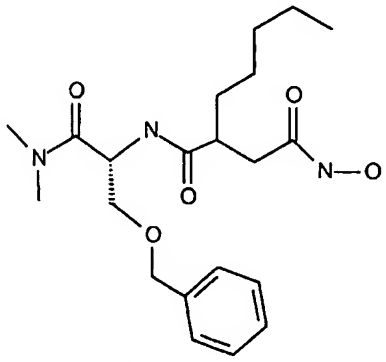


Compound 6

17. A pharmaceutical composition comprising a compound which inhibits peptide deformylase with the native iron catalytic metal center that is selected from the group consisting of:



Compound 1



Compound 2